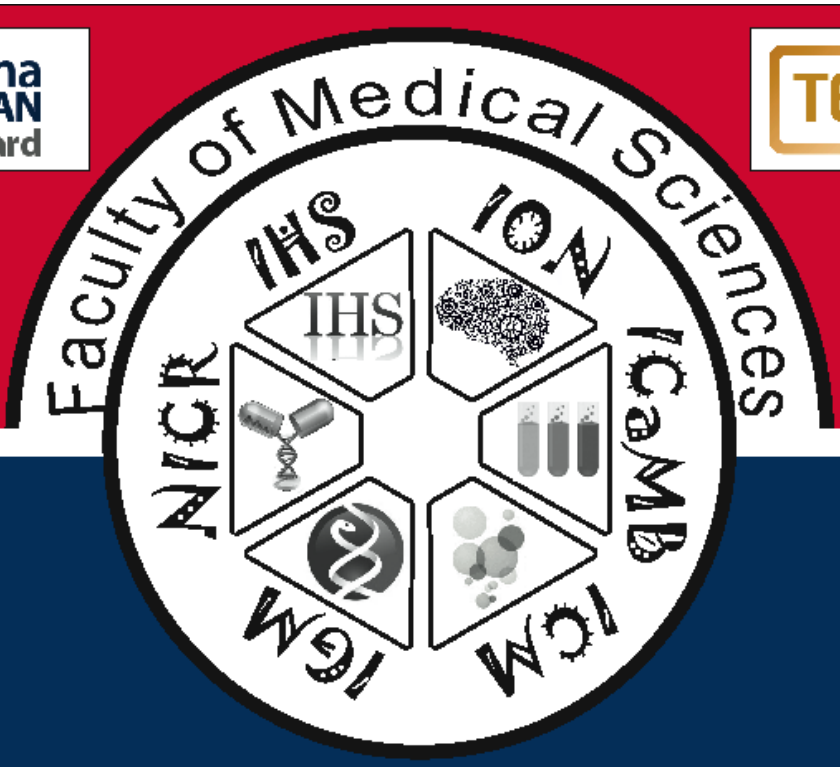
Athena SWAN Silver Award

TEF Gold



FMS - POst-doc Symposium

FPOS

1st Post-Doc Symposium

Newcastle, 15th June 2018

Newcastle University
Faculty of Medical Sciences

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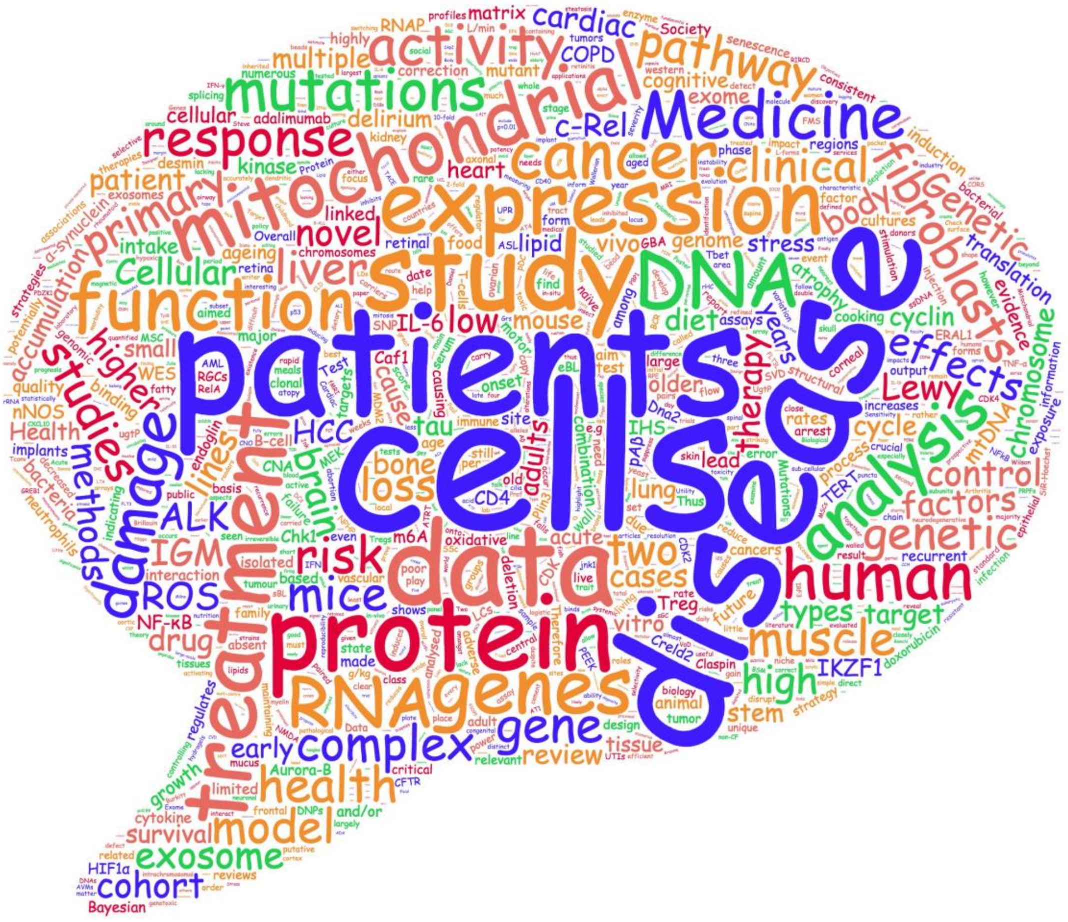
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FMS Post Doc Symposium 2018

This is the first Faculty of Medical Sciences Post-Doc Symposium to showcase the outstanding contributions and research of post-doctoral staff across all institutes. This first symposium is organised by the Post-Doctoral Committee newly-formed in 2017. We are delighted to have a keynote talk from **Prof. Steve Wilson**, Professor of developmental genetics at UCL, who will also present a **prize for best paper (including a £150 Amazon voucher).** Prizes will also be awarded for **best talk** and for **best poster**.

We have secured generous sponsorship from Cambridge Bioscience, Promega, Sigma, Invitrogen, Merck, Thermofisher Scientific, New England Biolabs, Diagenode and Active Motif, who will be providing material for the conference bags as well as sponsorship for the best poster, talk and paper prizes.

We hope you will enjoy the day!

You can vote for the best poster! Go to: www.menti.com, type in the code 628446 and put poster number!

FMS Post-Doctoral Committee

Established in 2017, we are here to organise events, provide support and information, and represent post-doctoral researchers' views to the faculty. Here you will find a list of your local institute representatives on the committee, as well as upcoming events, and important links to faculty resources.

We are continuously eager to hear from PDRAs from all institutes of FMS and open to shape the role of this committee in response to your needs, so please share your opinion via your local institute representatives, by emailing: fmspostdoccomm@newcastle.ac.uk, or using the contact form on our website. If you have some ideas or would like to join us, please also get in touch. We are always welcoming to new faces and new ideas!

FMS Post-Doctoral Committee

http://goo.gl/zeQmgN @fms_postdoccomm #nclfpos

Website: https://www.societies.ncl.ac.uk/fmspostdoccomm/

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FMS Post-Doctoral Committee (June 2018)

Acknowledgements

We would like to thank everyone who helped us to make this event happen.

- Prof Derek Mann – for the idea of a PostDoc focused event and the FMS for £5000 budget
- Kay Howes, Jill McKenna and Judith Williams - for advice and help in organisation
- Dr Paula Salgado and Dr Kevin Waldron – for assistance, advice and help
- All our sponsors without whom the prizes would not be so generous
- All the academics who helped with the best paper selection: Dr Kevin Waldron (ICaMB), Dr Paula Salgado (ICaMB), Prof Sophie Hambleton (ICM), Prof Simi Ali (ICM), Prof Helen Arthur (IGM), Prof Melissa Bateson (IoN) and Prof Craig Robson (NICR)
- Jeremy Domis (ICM) for being our photographer

1st FMS PostDoc Symposium Programme

08:30-09:30	**Registration**
09:30-09:40	**Welcome and Opening Remarks** (Urszula Cytlak-Chaudhuri and Derek Mann)
09:40-10:40	**Session 1: *Health, Disease & Vitality*** (chaired by Alex Egan and Julie Worrell)
09:40	L-form switching as a potential mechanism for the recurrence of urinary tract infection. **(Katarzyna Mickiewicz, ICaMB)**
09:55	Exercise dramatically improves age-related, inflammation-driven liver damage and cancer. **(Arianna Bianchi, ICM)**
10:10	The Utility of FLT3L as a Biomarker of Progenitor Cell Mass in Acute Leukaemia. **(Paul Milne, ICM)**
10:25	How do non-prescribed medicines (NPMs) contribute to polypharmacy in older adults with multimorbidity? **(Dapo Ogunbayo, IHS)**
10:40-11:00	**Coffee Break in the Atrium and David Shaw Foyer/Boardroom**
11:00-12:00	**Session 2: *Signalling, Development & Behaviour*** (chaired by Thomas Carle and Marina Garcia-Macia)
11:00	Site specific ROS signalling regulates stress adaptation through activation of mitochondrial quality control. **(Filippo Scialo, ICaMB)**
11:15	PRPF31 retinitis pigmentosa is caused by disrupted alternative splicing in cellular adhesion and ciliogenesis genes. **(Valeria Chichagova, IGM)**
11:30	A mixed methods programme of study on the determinants and outcomes of home food preparation. **(Susanna Mills, IHS)**
11:45	Heterozygous Glucocerebrosidase Mutations and Lipid Metabolism defects in Lewy Body disease. **(Marzena Kurzawa-Akanbi, IGM)**
12:00-12:30	**Flash Poster Presentations: 2-Minutes Each (Selected Posters displayed in Atrium)** (chaired by Kate Best and Diana Umeton)
12:30-14:00	**Lunch and Poster Session (Boardroom, Atrium and Sponsors' Foyer)**
14:00-15:00	**Keynote Speaker: Prof. Steve Wilson (UCL)** (chaired by Adrian Santos) Breaking symmetry in the brain - from genes to circuits and behaviour.

15:00-15:05 **Paper Prize Announcement by Prof. Steve Wilson**

15:05-15:30 **Best Post-Doc Paper Prize Talk: Vivek Nityananda (IoN)**

15:30-16:00 **Coffee Break (Boardroom)**

16:00-17:00 **Session 3: *Structures, Functions & Modelling***
(chaired by Martyna Pastok and Monika Olahova)

16:00 Development of the Caf1 protein as a multi-functional biomaterial for use in 3D cell scaffolds. **(Daniel Peters, ICaMB)**

16:15 Variants in EXOSC9 disrupt the RNA exosome and result in cerebellar atrophy. **(David Burns, IGM)**

16:30 Mitochondrial DNA deletions originate as a subcellular perinuclear niche in human skeletal muscle. **(Amy Vincent, IoN)**

16:45 Modelling localised doxorubicin-eluting bead therapy in combination with inhibition of DNA-PK in liver cancer. **(Catherine Willoughby, NICR)**

17:00-17:30 **Poster and Talk Prizes & Closing Remarks**

17:30-19:00 **Drinks Reception (Boardroom)**

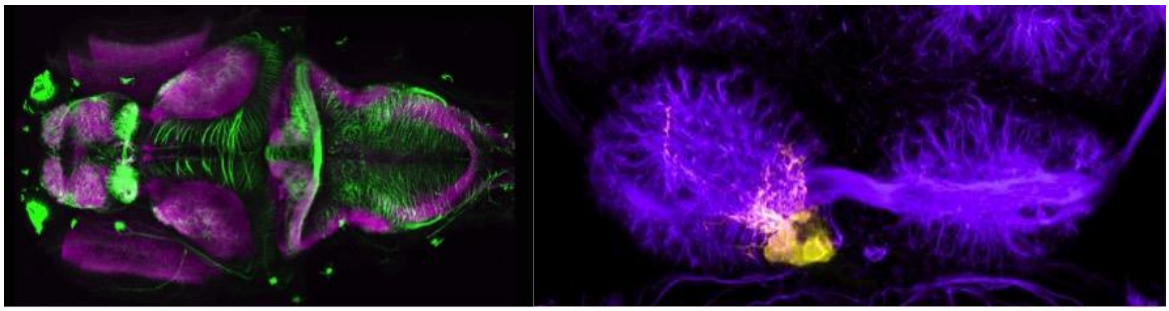
Keynote Speaker – Professor Steve Wilson:

Brain asymmetry – from genes to circuits and behaviour

Steve Wilson is Professor of Developmental Genetics and Vice-dean for Research at UCL in London. Ever since his post-doc at the University of Michigan with Steve Easter, his research has been focused on brain development using zebrafish as a model system with multiple high impact publications in Nature, Nature Neuroscience, PNAS, and Neuron.

Abstract

It is likely that the nervous systems of all bilaterally symmetric animals are left-right asymmetric with respect to processing of information and control of behaviour. However, we know very little about how asymmetries arise in development, how they are encoded in circuits and what their importance is for nervous system function. We use developmental, genetic, imaging and behavioural approaches to study habenular asymmetry in zebrafish to address these issues. One focus is to determine the mechanisms that lead to neurons on the left and the right acquiring different character and establishing different circuit connectivity between left and right sides of the brain. We have also been using optogenetic approaches to characterise functional properties on neurons on left and right and assessing how genetic mutations affecting laterality affect circuitry. In parallel we, and our colleagues, are developing behavioural assays to assess how circuit asymmetry affects behaviour.



Best Paper winner - Vivek Nityananda (IoN):

A Novel Form of Stereo Vision in the Praying Mantis

(Current Biology 2018, Vol 28, pages 588-593)

DOI link: (https://doi.org/10.1016/j.cub.2018.01.012)

Vivek Nityananda has a PhD from the Indian Institute of Science, Bangalore. He has since worked at the University of Minnesota and Queen Mary University of London. He has held a Marie Curie Research Fellowship, a Human Frontiers Science Program Fellowship and a fellowship at the Wissenschaftskolleg zu Berlin. He is currently a research associate at the Institute of Neuroscience where he researches stereo vision in the praying mantis. He has also worked towards engaging the public with research and was awarded a public engagement fellowship from the Great North Museum, Newcastle and a Wellcome Trust Small Arts Award to support these efforts.

Abstract

Stereopsis is the ability to estimate distance based on the different views seen in the two eyes. It is an important model perceptual system in neuroscience and a major area of machine vision. Mammalian, avian, and almost all machine stereo algorithms look for similarities between the luminance-defined images in the two eyes, using a series of computations to produce a map showing how depth varies across the scene. Stereopsis has also evolved in at least one invertebrate, the praying mantis. Mantis stereopsis is presumed to be simpler than vertebrates', but little is currently known about the underlying computations. Here, we show that mantis stereopsis uses a fundamentally different computational algorithm from vertebrate stereopsis—rather than comparing luminance in the two eyes' images directly, mantis stereopsis looks for regions of the images where luminance is changing. Thus, while there is no evidence that mantis stereopsis works at all with static images, it successfully reveals the distance to a moving target even in complex visual scenes with targets that are perfectly camouflaged against the background in terms of texture. Strikingly, these insects outperform human observers at judging stereoscopic distance when the pattern of luminance in the two eyes does not match. Insect stereopsis has thus evolved to be computationally efficient while being robust to poor image resolution and to discrepancies in the pattern of luminance between the two eyes.

Talks

O1

Katarzyna Mickiewicz

Title: L-form switching as a potential mechanism for the recurrence of urinary tract infection

Institute of Cell and Molecular Biosciences (ICaMB)

In this study, we employed modern molecular methods combined with patient histories to examine the possible involvement of cell wall deficient, “L-form” bacteria and their ability to revert to a walled state in recurrent urinary tract infections (UTIs).
Urine samples obtained every two weeks over a period of 6 months from a cohort of 30 elderly patients with recurrent UTIs were assessed for the presence of polymorphic bacteria by direct microscopy and by filtration. Fluorescence in-situ hybridization with DNA probes against the bacterial 16S rRNA was used to test the identity of the polymorphic structures. Isolated bacteria were assessed by time lapse microscopy for their ability to transform into L-forms and return to the walled state.
We demonstrate the existence of cell-wall deficient bacteria in fresh urine from 29 out of 30 patients with recurrent UTIs. Two different E. coli strains isolated from one of the patients, which reoccurred in this patient following fosfomycin treatment, were shown in real time to undergo an efficient transition from rod to L-form and back again. While in the L-form state, the bacteria were resistant to cell wall targeting antibiotics. L-forms switching of the isolated bacteria during fosfomycin treatment was reconstructed in urine. We propose that the recurrence of UTIs in elderly patients may, at least in part, be due to the persistence of L-form bacteria during treatment with antibiotics targeting the bacterial cell wall and their reversion to walled forms following antibiotic withdrawal.

Keywords: UTI; L-forms; recurrent infections

O2

Arianna Bianchi

Title: Exercise dramatically improves age-related, inflammation-driven liver damage and cancer

Institute of Cellular Medicine (ICM)

Whilst there are several mouse models to study the potential of therapeutic interventions in chronic liver disease (CLD) and hepatocellular carcinoma (HCC) most lack one of the major risk factors associated with HCC development 'ageing'. Ageing is associated with chronic low-grade inflammation, increased hepatic lipid accumulation, loss of the regenerative potential of hepatocytes and an increased risk of fibrosis. In this study we are utilizing the aged NF-kB1-/- model characterised by spontaneous premature ageing, systemic progressive low-grade inflammation, hepatic steatosis, fibrosis and HCC to investigate the impact of a lifestyle therapy 'moderate exercise' on age-related CLD and HCC development.
16 month old NF-kB1-/- with established chronic inflammation and spontaneous liver damage were treadmill exercised 3 times/week 20cm/sec or remained as sedentary controls. Behavioural tests were performed at 16, 17 and 18 months. Animals were sacrificed at 19 months and serum and organs removed for IHC analysis, cytokine array and liver enzyme and lipid quantification.
Development of spontaneous liver disease including inflammation, steatosis and liver tumours was dramatically reduced in the exercise group. IHC analysis revealed a striking reduction in various immune cells in the exercised livers. Cytokine array in serum and liver protein revealed a significant reduction in the chemokines CXCL9 and CXCL10 in both blood and liver from the exercised group. A reduction in steatosis shown by H&E and circulating LDL and cholesterol in the exercised mice was also observed. Furthermore, activity tests revealed that exercise positively impacts locomotor activity of the mice.
We have shown that a simple moderate exercise regime in an aged group of mice with chronic low grade inflammation and CLD significantly improves liver health and the development of cancer. This striking finding has major implications as an easily translatable low impact therapy for an increasingly aged population with increasing NASH and HCC numbers.

Keywords: exercise; ageing; chronic inflammation; liver disease

O3

Paul Milne

Title: The Utility of FLT3L as a Biomarker of Progenitor Cell Mass in Acute Leukaemia.

Institute of Cellular Medicine (ICM)

Current methods for diagnosing and monitoring patients with acute myeloid leukaemia (AML) involve invasive bone marrow aspiration and costly genetic molecular tests which require individualisation and are not universally applicable. Molecular monitoring of acute leukaemia is a top priority around the world but no-one to our knowledge has identified a simple biomarker that could potentially replace these costly and invasive tests.

FLT3-Ligand is a cytokine which has an important role in the haematopoiesis of myeloid cells. AML cells express high levels of the FLT3 receptor and carry high rates of mutation within the FLT3 receptor gene. However, the levels of FLT3-Ligand have never been examined in AML at diagnosis.

Eighty patients with AML were recruited at diagnosis. Low or absent serum FLT3-Ligand identified patients with acute leukaemia compared with normal controls and pre-leukaemic syndromes (sensitivity and specificity above 94%). Successful remission after chemotherapy was associated with a normalisation of FLT3-Ligand levels. FLT3-Ligand levels remained low or absent in patients who failed chemotherapy induction or had refractory disease. Importantly, this was seen weeks before conventional testing was able to diagnose treatment failure. In patients in which FLT3-Ligand levels normalised after the first round of chemotherapy, the 5-year overall survival rate was 100%, compared to 40% of patients who took longer to normalise or where FLT3-Ligand remained absent. In patients who have received bone marrow transplantation, FLT3-Ligand dropped preceding relapse, up to 140 days prior to clinical diagnosis of relapse.

It is thus feasible to monitor patients weekly with FLT3-Ligand to identify disease status rather than relying on bone marrow aspirations which can only be performed every 1-2 months. Additionally, FLT3-Ligand is easily measurable in serum for about £6 per test compared with £500 for full bone marrow evaluation and up to £5,000 for molecular disease monitoring.

Keywords: AML; Biomarker; FLT3L

O4

Dapo Ogunbayo

Title: How do non-prescribed medicines (NPMs) contribute to polypharmacy in older adults with multimorbidity?

Institute of Health and Society (IHS)

With increasing life expectancy, the number of people living with chronic health conditions is also on the increase. Multi-morbidity, the co-occurrence of two or more chronic conditions, is increasingly common, especially among older adults (≥65 years). Most older adults with multi-morbidity require one or more medicines to live well and manage their health. Polypharmacy, which is the concurrent use of multiple medicines by one individual (usually five or more) can be problematic, especially among older adults with multi-morbidity. Polypharmacy potentially increases the risks of non- adherence to treatment, drug toxicity and interactions, poor quality of life, hospitalisations and death.

The use of prescribed medicines is the main predictor of polypharmacy among older adults. There are however concerns about the use of non-prescribed medicines (NPMs) obtained via self-medication with over-the-counter, herbal and other medicines (e.g. those bought over the internet) that have not been prescribed. The adverse consequences of polypharmacy associated with NPMs may be even greater for older, frail adults who are already on numerous prescribed medicines and experiencing cognitive and functional impairments and other geriatric syndromes.

This on-going programme of work aims to understand the contribution of NPMs to polypharmacy among older adults. The first work-stream is a systematic literature review aimed at identifying and synthesising current evidence in order to characterise/define NPMs-use among older adults, determine its prevalence, and outline predisposing factors and outcomes. The second work-stream involves secondary data analyses of population level data using the Cognitive Function and Ageing Studies (CFAS) datasets. The analyses will explore patterns and factors associated with NPM use and variables such as socio-demographic, disease and health status and health service use.

This presentation will provide early findings from the research programme of work and discuss implications for clinicians and policymakers on developing strategies that go beyond review of prescribed medicines.

Keywords: ageing; multi-morbidity; polypharmacy; medicines-use

O5

Filippo Scialo

Title: Site specific ROS signalling regulates stress adaptation through activation of mitochondrial quality control

Institute of Cell and Molecular Biosciences (ICaMB)

Reactive Oxygen Species (ROS) have been extensively studied during the last 50 years but still much is unknown about how they operate in vivo. Although, ROS can cause oxidative damage, they are also essential cellular messengers required for maintaining cellular homeostasis. Moreover, boosting ROS levels extends lifespan in several animal species. The present consensus is that the amount of ROS determines their physiological effects with low levels of ROS having beneficial effects, whereas higher levels being detrimental. Here we demonstrate that the place where ROS are generated is instrumental in determining their physiological effects. We show that respiratory complex I (CI) produces ROS both in forward and in reverse direction in the Drosophila brain. However, only ROS generated via Reverse Electron Transport (RET) are required for stress adaptation. Accordingly, suppression of RET attenuates the transcriptomic response to stress and dramatically shortens lifespan. Next, we show that inactivation of RET-ROS prevents mitochondrial fission causing the accumulation of respiratory-deficient mitochondria. Surprisingly, maintaining low levels of ROS activates Target of rapamycin (Tor) signalling and subsequently loss of proteostasis that have been shown in old individuals with high levels of mitochondrial ROS. Interestingly, we show that only restoring mitochondrial turnover restores Tor signalling and survival under stress. In summary, our results demonstrate the importance of site-specific ROS signalling in maintaining both mitochondrial function and cellular homeostasis. We therefore propose the manipulation of RET-ROS as a new therapeutic strategy to delay ageing and prevent the onset of age-related diseases.

Keywords: ROS; signalling; mitochondria; ageing

O6

Valeria Chichagova

Title: PRPF31 retinitis pigmentosa is caused by disrupted alternative splicing in cellular adhesion and ciliogenesis genes

Institute of Genetic Medicine (IGM)

Retinitis pigmentosa (RP) is one of the most common forms of inherited blindness with more than 1 million people affected worldwide. It remains a medically challenging disease with no effective treatments. Mutations in pre-mRNA processing factors (PRPFs) cause 40% of autosomal dominant RP, but it is unclear why mutations in ubiquitously expressed PRPFs cause retinal-specific degeneration. In order to elucidate the molecular basis of the effects of PRPF mutations on various retinal cell types, we generated RP type 11 (PRPF31-mutated) patient-specific retinal organoids and retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSC). Impaired alternative splicing of genes encoding pre-mRNA splicing proteins occurred in patient-specific retinal cells and Prpf31+/- mouse retinae, but not in fibroblasts and iPSCs, providing supporting evidence to retinal-specific phenotypes of PRPFs. Amongst retinal cell types, RPE was the most affected showing a number of morphological and functional abnormalities, including loss of apical-basal polarity, reduced trans-epithelial resistance, phagocytic capacity, microvilli, and cilia length and incidence. Neural retina was also affected displaying disrupted cilia morphology and features of cell stress. We subsequently corrected the PRPF31 mutation in cells derived from a patient with the most severe clinical phenotype and showed rescuing of key structural and functional phenotypes in RPE and photoreceptors, providing proof-of-concept for future therapeutic strategies.

Keywords: retinitis pigmentosa; iPSCs; cilium; retinal organoids

O7

Susanna Mills

Title: A mixed methods programme of study on the determinants and outcomes of home food preparation

Institute of Health and Society (IHS)

Diet-related diseases are the greatest cause of morbidity and mortality worldwide. Food preparation methods are linked to diet and health. The aim of this study was to explore the determinants and outcomes of home food preparation, using mixed methods.

The first research phase was a systematic review of observational studies on the health and social determinants and outcomes of home cooking. Key determinants included female gender, greater leisure time availability, close personal relationships, and culture and ethnicity. Putative outcomes were mostly at an individual level and focused on potential dietary benefits.

The second phase involved qualitative interviews exploring home food preparation practices, experiences and perceptions amongst adults from the United Kingdom (UK). Key emergent themes concerned the cook (identity), task (process of cooking) and context (situational drivers). Practices changed over the life course and reflected compromises between varied competing demands. Comparison with focus group data from Baltimore, United States, showed that 'home cooking' was distinct from other types of cooking at home. 'Home cooking' was defined as: preparing a meal from scratch; cooking with love and care; and nostalgia and was not aligned closely with principles of healthy eating.

The third phase comprised analyses of cross-sectional data on participants' meal consumption patterns, sociodemographics, diet and markers of cardio-metabolic health, from a large population-based UK cohort study. Eating home cooked meals more frequently was significantly associated with being female, older, of higher socioeconomic status and not working overtime. Varying patterns of association were observed for consuming takeaways, ready meals and meals out. Eating home cooked meals more frequently was significantly associated with a range of healthier dietary indicators, and lower adiposity.

Overall, preparing and eating meals cooked at home were found have complex and varied determinants, and to offer a range of putative benefits, indicating potential to enhance the public's health.

Keywords: diet; nutrition; cooking; mixed methods

O8

Marzena Kurzawa-Akanbi

Title: Heterozygous Glucocerebrosidase Mutations and Lipid Metabolism defects in Lewy Body disease.

Institute of Genetic Medicine (IGM)

Heterozygous mutation of the Glucocerebrosidase (GBA) gene is linked to a greater risk of developing Parkinson's disease (PD) and dementia with Lewy bodies (DLB), common forms of age-related neurodegeneration characterized by accumulation of alpha-synuclein protein in Lewy body aggregates within the brain. GBA plays a role in the metabolism of specific lipids by cells, and accumulation of lipids occurs in individuals with homozygous GBA mutation in Gaucher's disease. We hypothesized that lipid metabolism is altered in nerve cells due to GBA mutations and these changes predispose towards alpha-synuclein accumulation in PD/DLB.

To test this hypothesis, we performed global lipid analysis of the cingulate cortex (brain area significantly affected by alpha-synuclein pathology) and frontal cortex (brain area with a low neuropathological burden) in GBA mutation carriers and non-carriers among PD/DLB and controls. We also purified exosomes (small extracellular vesicles that carry molecules) from post-mortem cerebrospinal fluid (CSF) and tested those for lipid composition and presence of alpha-synuclein.

Our study identified sphingolipids changes in brains from all PD/DLB patients compared to controls. The most prominent changes were found in ceramides, a family of biologically active lipids. Changes in ceramides specific to PD/DLB with GBA mutations were not seen, indicating that these changes are a characteristic marker of all types of PD and DLB. Importantly, exosomes purified from PD/DLB CSF were heavily loaded with ceramides, suggesting a role in the disease process. Alpha-synuclein protein was also found attached and present inside exosomes. Furthermore, we found that by using exosomes and a novel protein aggregation method, we were able to detect alpha-synuclein pathology (aggregation), even at very low levels.

Future work will involve an in-depth analysis of sphingolipid metabolism and its effect on alpha-synuclein aggregation intracellularly and within exosomes. These approaches will produce a basis for developing therapies and diagnostic method(s) for PD/DLB.

Keywords: Lewy body disease; glucocerebrosidase; lipids; exosomes

O9

Daniel Peters

Title: Development of the Caf1 protein as a multi-functional biomaterial for use in 3D cell scaffolds.

Institute of Cell and Molecular Biosciences (ICaMB)

The bacterium responsible for the plague known as the Black Death protects itself from the immune system by surrounding itself with a non-stick capsule. The capsule is made of a protein called Caf1, which has a unique combination of interesting properties that make it useful as a multi-functional biomaterial. It has a very high thermostability (>90°C) that is retained even in harsh chemical conditions, it can be up to µm in length, and cells adhere to it very poorly in vitro. Critically, the protein can be expressed recombinantly in E. coli, and therefore its properties can be further engineered through standard DNA modification techniques. Additionally, the protein can be induced to form 3D porous hydrogels through the use of chemical cross-linkers, is free from animal products, and is compatible with 3D printing technologies. Here, we show the development of Caf1 as a biomaterial, including the engineering of a cell adhesion motif into Caf1 that reverses its non-stick property and allows attachment of U2OS cells; the construction of 3D Caf1 hydrogels of different stiffness and porosity; and the generation of mosaic Caf1 polymers where two types of Caf1 subunit are made at once, expanding its range of functionalities. The production of thermostable, animal-free hydrogels of definable bioactivity has many applications both in biomedicine and beyond, with initial applications in 3D cell scaffolds for drug discovery and wound healing being actively investigated.

Keywords: Biomaterials; Protein Engineering; 3D Tissue Culture

O10

David Burns

Title: Variants in EXOSC9 disrupt the RNA exosome and result in cerebellar atrophy

Institute of Genetic Medicine (IGM)

The exosome is a conserved multi-protein complex which is essential for correct RNA processing. Recessive variants in exosome components EXOSC3, EXOSC8, and RBM7 cause various constellations of pontocerebellar hypoplasia (PCH), spinal muscular atrophy (SMA), and central nervous system demyelination. Here we report four unrelated affected individuals with recessive variants in EXOSC9 and study the effect of the variants on the function of the RNA exosome in vitro in affected individual's fibroblasts and skeletal muscle and in vivo in zebrafish. The clinical presentation was severe, early-onset, progressive SMA-like motor neuronopathy, and cerebellar atrophy, and in one affected individual congenital fractures of the long bones. Three affected individuals of different ethnicity carried the homozygous c.41T>C; p.(Leu14Pro) variant, while one affected individual was compound heterozygous for c.41T>C; p.(Leu14Pro) and c.481C>T; p.(Arg161*). We detected reduced EXOSC9 protein in fibroblasts and skeletal muscle, and a reduction of the whole multi-subunit exosome complex was observed on blue-native polyacrylamide gel electrophoresis. RNA sequencing of fibroblasts and skeletal muscle detected significant >2-fold changes in genes involved in neuronal development, cerebellar and motor neuron degeneration demonstrating the widespread effect of the variants. Morpholino oligonucleotide knockdown and CRISPR/Cas9 mediated mutagenesis of exosc9 in zebrafish recapitulated aspects of the human phenotype, as it has in other zebrafish models of exosomal disease. Specifically, portions of the cerebellum and hindbrain were absent and motor neurons failed to develop and migrate properly. In summary, we show that variants in EXOSC9 result in a neurological syndrome combining cerebellar atrophy and spinal motoneuronopathy, thus expanding the list of human exosomopathies.

Keywords: neurodegenerative diseases; RNA exosome; cerebellar atrophy

O11

Amy Vincent

Title: Mitochondrial DNA deletions originate as a subcellular perinuclear niche in human skeletal muscle

Institute of Neuroscience

Defects of mitochondrial DNA (mtDNA) maintenance are an important cause of mitochondrial disease causing multiple mtDNA deletions in adults. In these patients mtDNA deletions form sporadically and clonally expand within individual muscle fibers causing respiratory chain deficiency. To understand the progressive nature of these diseases we need to understand how these mutations clonally expand. Using sub-cellular molecular analyses and imaging of single human skeletal muscle fibers we find that mtDNA deletions first exceed the biochemical threshold causing biochemical deficiency in focal regions adjacent to the myonuclei. Perinuclear foci containing mtDNA deletions show local elevations of both mitochondrial mass and mtDNA copy number reflecting mitochondrial proliferation. Accordingly, focal expansion of mtDNA deletions is associated with a local increase in mitochondrial biogenesis and unfolded protein response (UPRmt) signaling pathways specifically in sub-cellular regions with cytochrome c oxidase (COX) deficient mitochondria. We also find that mitochondrial dysfunction physically spreads following the three-dimensional anisotropic architecture of the mitochondrial network. These sub-cellular resolution data provide new insights into the mechanisms underpinning clonal expansion of mtDNA deletions and progression of mitochondrial myopathy.

Keywords: clonal expansion; skeletal muscle; mitochondria

O12

Catherine Willoughby

Title: Modelling localised doxorubicin-eluting bead therapy in combination with inhibition of DNA-PK in liver cancer

Northern Institute for Cancer Research (NICR)

Transarterial chemoembolisation (TACE) is widely used in the treatment of hepatocellular carcinoma (HCC). However, TACE is rarely curative, and investigational approaches to improve treatment efficacy are limited by the requirement for large animal models. In HCC patients, increased expression of the key DNA repair enzyme DNA-dependent protein kinase (DNA-PK) correlates with resistance to TACE, advanced tumour grade and shorter survival. We developed a murine model of drug-eluting bead therapy and explored selective inhibition of DNA-PK to enhance localised doxorubicin treatment in HCC using NDD0004 - a novel, orally-bioavailable inhibitor of DNA-PK catalytic activity.

NDD0004 was evaluated in combination with doxorubicin in human HCC cell lines (Hep3B, HepG2, Huh7). DNA-PK activity was determined by Ser2056 phosphorylation status, DNA damage quantified by γH2AX levels, cell proliferation determined by SRB assays and cell survival assessed using clonogenic assays. A model of localised chemotherapy was established by intra-tumoural injection of doxorubicin-loaded beads into subcutaneous Huh7 xenografts in CD1 nude mice. Oral twice-daily treatment with 30 mg/kg NDD0004 was started 1 hour following bead implantation and continued for ≤20 days.

NDD0004 dose-dependently inhibited activation of DNA-PK in HCC cell lines in vitro, and increased and sustained DNA damage following doxorubicin treatment. NDD0004 sensitised HCC cell lines to doxorubicin in proliferation and survival assays by approximately 5-fold. Combining NDD0004 with doxorubicin-loaded beads in vivo significantly inhibited the rapid growth of HCC tumours compared to monotherapy (Time to RTV4 = 18 versus 11 days, $p<0.01$, Mann-Whitney). Furthermore, γH2AX levels in cells surrounding doxorubicin-loaded beads were significantly increased by NDD0004 treatment ($p<0.0001$, ANOVA).

In conclusion, selective inhibition of DNA-PK sensitised HCC cell lines to doxorubicin in vitro and augmented the activity of localised chemotherapy in HCC xenografts in vivo. These data support the concept of combining a DNA-PK inhibitor with localised DNA-damaging therapies for HCC.

Keywords: liver, cancer, DNA-PK, doxorubicin, combination, therapy

Posters

Selected Flash Presentations:

P5 - Nuno Mendonca: Protein intake and disability trajectories in the very old: The Newcastle 85+ Study

P14 - Diana Papini: A potential new error correction mechanism for chromosome segregation in anaphase

P17 - Ricardo Gouveia: Brillouin spectro-microscopy as a new tool to explore stem cell niche biomechanics

P24 - Vinciane Siant-Criq: Identifying new therapeutic targets for Cystic Fibrosis airway disease

P25 - Ella Dennis: The role of a novel protein folding chaperone, CRRELD2, in skeletal development and disease

P27 - Helen Griffin: A Multiomic Approach to Identify Genetic Modifiers of Reversible Infantile Respiratory Complex Disorder

P35 - Fei Gao: Signalling pathway involved with monitoring mitochondrial ribosomal stress

P36 - Jill Hunter: Regulation of checkpoint kinase signaling and tumourigenesis by the NF-kB regulated gene, CLSPN

P50 - Abeer Dannoura: GREB1 and PDZK1 predict oestrogen responsiveness in gynaecological cancer

P59 - Peixun Zhou: Investigation of FOXO1 Abnormalities in Paediatric Burkitt Lymphoman

P60 - Henrique de Paula-Lemos: Dissecting the immunoresponses induced by systemic activation of the Stimulator of Interferon Genes (STING)

P64 - Fiona Malcomson: Adherence to the WCRF/AICR cancer prevention recommendations and WNT pathway-related markers of bowel cancer risk

P1

Nicola Simcock

Title: Understanding honeybee (*Apis mellifera*) taste using Drosophila as a novel expression system.

Institute of Neuroscience

Honeybees are our primary insect pollinator, globally contributing billions (£'s) to the agricultural industry each year through pollination services. Chemosensation is vital for identifying appropriate floral food sources and while the vast majority of bee research is devoted to olfaction, alarmingly little is known about the function of the gustatory system. Interestingly, the honeybee possesses the fewest number of gustatory receptors (Grs) of any animal annotated to date. This discovery poses an interesting conundrum: how to navigate the numerous components of nectar and pollen, including toxins, with such a simple system? Additionally, the honeybee provides a useful alternative to understanding the function of an entire gustatory system, over the complexity of other insect models. However, as a eusocial insect, with a majority of sterile female workers, genetic manipulation has always posed a problem in honeybee research. Here, we developed a novel approach using Drosophila as a heterologous expression system to determine honeybee Gr function. Using the relatively new Drosophila 'empty neuron' system, developed in the Amrein lab (Texas A&M), we expressed all honeybee Grs (both singly and in pairs) to investigate function using calcium imaging. Preliminary results suggest that, despite structural similarity, honeybee Grs have a broader range of chemical ligands than their closest orthologs. Unsurprisingly, honeybee gustation appears primed toward sugar sensing, but this work offers an interesting new insight into potential receptor redundancy and the importance of specific nutrient detection.

Keywords: gustation; honeybee; Drosophila; heterologous expression system

P2

Kirsty McAleese

Title: Investigating the pathogenesis of white matter damage in Alzheimer's disease

Institute of Neuroscience

Introduction: Accurate diagnosis between vascular dementia (VaD) and Alzheimer disease (AD) is difficult as symptoms can greatly overlap. The white matter (WM) consists of axons wrapped in myelin, which may become damaged as a result of a brain-blood vessel disease called small vessel disease (SVD). Evidence of WM damage on MRI brain scans is assumed to be linked to SVD and will sway a diagnosis towards VaD. However, research indicates WM damage in AD can be linked to a mechanism of axonal destruction called Wallerian degeneration triggered by AD protein deposits. Therefore, SVD cannot be assumed to be the only cause of WM damage as it may in fact be AD, and the misdiagnosis of patients can be detrimental.
This study aimed to investigate the composition of WM damage in AD, and to determine the aetiology of WM damage in AD.
Methods: Human brain tissue from 55 age-matched donors: AD, n=27; non-demented, n =28 (WM damage due to SVD). We microscopically measured the severity of the WM damage, the amount of myelin and axonal loss, AD-associated protein deposits, severity of vessel disease, as well as biochemical markers of Wallerian degeneration and vessel disease.

Results: WM damage in AD donors had significantly higher axonal loss compared to non-demented donors that primariliy exhibited myelin loss. AD tissue also had a significantly increased amount of Wallerian degeneration marker. In the non-demented donors, WM damage was linked to SVD severity and had a significant increase in an ischemia marker.

Conclusion: WM damage in AD is different to that seen in normal ageing and is linked to a much higher loss of axonal connections and is caused by a different mechanism of axonal destruction. These findings are critical for the accurate interpretation of MRI characteristics and differentiation of AD and VaD.

Keywords: White matter; Alzheimer's disease; dementia; neurodegeneration

P3

Aaron Gardner

Title: Linking cystic fibrosis lung disease pathophysiology with ceramide accumulation and inflammation

Institute for Cellular Medicine (ICM)

The genetic and functional basis of cystic fibrosis (CF) is well described. However, the exact pathogenesis of CF lung disease (CFLD), the major cause of morbidity and mortality, remains an area of great interest and investigation.

Several previous studies have demonstrated an accumulation of the sphingolipid ceramide in airway tissue from people with CF, and in CF murine models. Ceramide is a key lipid component of the plasma membrane but also plays an important role in numerous cellular signalling pathways. Accumulation of ceramide has been linked with the development of inflammation in several disorders, including CF; although the mechanism linking CFTR dysfunction with ceramide accumulation and its role in driving inflammation remains unknown.

Using differentiated air liquid interface (ALI) cultures of primary human bronchial epithelial cells (PBECs) from people with CF and non-CF controls, we aim to understand the links between CF, ceramide accumulation and inflammation.

Ceramide was elevated in CF PBEC-ALI cultures compared to non-CF controls as assessed mass-spectrometry. This elevation was driven by deregulation of the key enzymes sphingomyelinase, which catalyses the breakdown of sphingomyelin into ceramide, and ceramidase which converts ceramide to sphingosine, at the functional, protein and mRNA level. This accumulation of ceramide correlated with increased secretion of pro-inflammatory cytokines such as IL-8 and IL-1b, as well as increased susceptibility to bacterial infection.

Application of recombinant human ceramidase (rHC) to ALI cultures normalised ceramide levels in CF cultures to those observed in non-CF controls. This application of rHC significantly reduced the secretion of IL-8 and IL-1b from CF cultures towards baseline for up to 5 days. Furthermore, rHC treatment also significantly reduced the susceptibility of CF cultures to bacterial infection, an effect which may be mediated by increased production of sphingosine, which has demonstrated anti-bacterial characteristics.

Keywords: cystic fibrosis; lung; inflammation; infection; therapeutic

P4

Suzannah Harnor

Title: Design and Synthesis of Allosteric Inhibitors of Cell Cycle Proteins as Potential Cancer Therapeutics

Northern Institute for Cancer Research (NICR)

Cyclin-dependent kinases are key regulators of cell cycle progression and transcription, with functionality that is strictly dependent on association with their partner proteins, the cyclins.1 Cyclin E1 is overexpressed in many breast cancers, and interaction with CDK2 has been linked to unfavourable patient prognosis. Recent studies have documented high cyclin E1 protein expression in ovarian cancer, osteosarcoma, non-small cell lung cancer (NSCLC), bladder, oesophageal, colorectal, and gastric cancer. In high-grade serous ovarian cancer (HGSC) cyclin E1 (CCNE1) amplification occurs in approximately 20% of patients.2 This is clinically associated with poor overall survival and primary treatment failure. CDK inhibitors currently in clinical trials exclusively target the ATP site and suffer from issues of selectivity and associated off-target toxicity. There is, therefore, a significant need to identify novel ways to interfere with CDK/cyclin function. Recent research has revealed a hydrophobic pocket in CDK2 that is independent of the ATP site. Compounds that bind to this allosteric site cause structural rearrangements rendering the enzyme unable to interact with cyclin E1.4 The CDK2 allosteric site offers an alternative route to inhibition that has the potential to lead to a new class of anticancer drug, potentially avoiding the off-target effects seen with current CDK inhibitors. A fragment-based screen was carried out and compound 1 was shown to bind to an allosteric site of CDK2.6 Structure-activity relationship studies have been conducted to improve the physical properties of this hit and led to the identification of hydroxyl derivative 2. Further optimisation was carried out by replacing the propanol side chain with various amides and amines with a view to gain additional H-bond interactions to the protein, whilst maintaining good solubility in an attempt to prove the hypothesis that small molecule inhibition can disrupt the interaction between CDK2 and cyclin E.

Keywords: allosteric; CDK; cyclin; inhibitor

P5

Nuno Mendonca

Title: Protein intake and disability trajectories in the very old: The Newcastle 85+ Study

Institute of Health and Society (IHS)

Introduction: The very old (≥85 years and over) are the fastest growing age group in most western societies and are at increased risk of disability. It is projected that the percentage of very old in the UK that require 24-hour care will increase by 82% from 2010 to 2030. In addition, mean protein intake is lower in older adults than in younger populations. There is limited research exploring adequate protein intake and delayed disability onset.

Methods: Protein intake was estimated with 2x24-hr multiple pass recalls (24hr-MPR) at baseline. Disability was measured as difficulty performing 17 activities of daily living (ADL) at baseline, at 18, 36 and 60 months. Trajectories were derived through mortality-adjusted group-based trajectory modelling (GBMT). The analytical sample comprised of 722 participants and the effect of protein intake (g/kg adjusted body weigh/day [g/kg aBW/d]) on disability trajectories was examined by multinomial logistic regression.

Results: Four disability trajectories between the age of 85 and 90 were derived: AT1, a constant very low disability trajectory; AT2, low increasing to mild; AT3, mild increasing to moderate; and AT4, moderate increasing to severe disability. Each unit increase in protein (g) per kg aBW/d was associated with higher odds of AT1 rather than AT4 over 5 years in models adjusted for key covariates (OR: 6.35, 95%CI: 1.53-26.3). Participants with protein intake ≥1.0 g/kg aBW/d (but not 0.8 g/kg aBW/d) were three times more likely to belong to AT1 than to AT4 (OR: 3.06, 95%CI: 1.33-7.06).

Conclusion: Higher protein intake, especially above 1.0 g/kg aBW/d, might protect against disability in the very old.

Keywords: protein; disability; very old; malnutrition

P6

Marina Garcia Macia

Title: Autophagic approaches to prevent fatty liver disease

Institute for Cellular Medicine (ICM)

Accumulation of lipid droplets (LDs) can lead to diseases such as non-alcoholic fatty liver disease, which is the most frequent liver pathology in western countries. The perilipin (Plin) family are the group of proteins that coat LDs, controlling their biogenesis, stabilization, and preventing their degradation. Plin3 function appears to be important in the initial stages of the LD maturation and is a marker of early stage of fatty liver disease. Recent studies have revealed that autophagy is involved in LD degradation and, therefore, may be crucial to avoid lipid accumulation. Autophagy gets activated after fatty acid treatment, however mechanisms involved in this activation are poorly understood. We identified here an important role of Plin3 in the selective degradation of lipid droplets after oleic acid treatment in fibroblasts and primary hepatocytes. Our results show how LC3-II and Lamp1 get accumulated in the LD after lysosomal inhibitors treatment, therefore autophagy machinery is selectively recruited in the surface of the LD. Plin3 is a clear target of this specific autophagy as its expression increases when autophagy is inhibited, same results are found with the lysosomal inhibitors as with the Atg7 silencing. When Plin3 is silenced, autophagic machinery is reduced in the LD surface. However, macroautophagy doesn't seem to be altered. Thus, our study indicates that Plin3 is crucial to get LDs degraded by autophagy and this may lead to the discovery of new clinical targets to prevent or treat fatty liver disease.

Keywords: autophagy; lipophagy; NAFLD; Plin3; lipid droplet

P7

Adrian Santos-Ledo

Title: Alternative splicing of jnk1 directs cardiac morphogenesis

Institute of Genetic Medicine (IGM)

Congenital heart defects (CHD) occur in one per cent of live born infants. Although both environmental and genetic causes are proposed human candidate genes remain elusive.
Our laboratory has been studying the non-canonical Wnt/Planar Cell Polarity (PCP) signalling and has shown it is essential for heart development. Disturbances of several genes within the pathway lead to outflow tract heart malformation in animal models. The c-Jun amino-terminal kinase (Jnk) gene is a highly conserved downstream component of the PCP pathway, which has not been shown to play a role in heart development although it participates in several other pathways implicated in CHD.
We set out to investigate the role of Jnk in development using zebrafish. These small tropical bony fish are a valuable laboratory model for the study of cardiac development. There is conservation of developmental processes and genes; the genome has been sequenced and a variety of genetic and imaging techniques are well established.
We first analysed the temporal and spatial expression of the zebrafish jnk genes using in-situ hybridisation and established presence of unique jnk1 transcripts within the zebrafish heart. Using both CrispR/Cas9 genome editing and morpholino knockdown we went on to demonstrate the role of jnk1 in cardiac development. Importantly the genome duplication in zebrafish allowed us to dissect the role of alternative spliced jnk1 transcripts and demonstrate requirement in both specification of ventricular cardiomyocytes and left right patterning.

Keywords: Jnk; Mapk; cardiovascular; left-right patterning; zebrafish

P8

Sophie Cassidy

Title: Objectively measured physical activity in 52,556 adults with cardio-metabolic disease: a UK Biobank study

Institute for Cellular Medicine (ICM)

Aim: Cardio-metabolic disease and physical activity are closely related but large-scale objective studies which measure physical activity are lacking. Using the largest accelerometer cohort to date, we aimed to investigate whether there is an association between disease status and accelerometer variables 5 years on.

Methods: 106,053 UK biobank participants wore a wrist-worn GENEactiv monitor. Those with acceptable wear time (>3 days), were split into 4 cardio-metabolic disease groups based on self-report disease status which was collected 5±1 years prior. Multiple linear regression models were used to investigate associations, controlling for confounders and stratified for gender.

Results: Average daily acceleration was lower in men ('healthy'-42±15mg v 'Type 2 diabetes + cardiovascular disease (CVD)'-31±12mg) and women ('healthy'-44±13mg v 'Type 2 diabetes + CVD'-31±11mg) with cardio-metabolic disease and this was consistent across both week and weekend days. Men and women with the worst cardio-metabolic disease perform around half of moderate to vigorous physical activity on a daily basis compared to healthy individuals and spend almost 7 hours per day in 30min Inactivity bouts. Significant associations were seen between cardio-metabolic disease and accelerometer variables 5 years on when controlling for confounders.

Conclusion: In the largest accelerometer cohort to date, there are significant associations between cardio-metabolic disease and physical activity variables after 5 years of follow up. Tri-axial accelerometers provide enhanced measurement opportunities for measuring lifestyle behaviours in chronic disease.

Keywords: epidemiology; devices; lifestyle

P9

Nina Wilkinson

Title: Personalising adalimumab treatment of patients with psoriasis: a UK, multi-centre, longitudinal observational cohort study

Institute of Health and Society (IHS)

Biological therapy has revolutionised the management of moderate to severe psoriasis, but not all patients respond and some lose response over time. Drug levels, which are strongly influenced by anti-drug antibodies (ADA), consistently correlate with treatment outcomes across multiple inflammatory diseases with limited data in psoriasis. The aim of this study was therefore to investigate the clinical utility of therapeutic drug monitoring to optimise outcomes in psoriasis, using the exemplar TNF antagonist, adalimumab.
Adults enrolled onto the British Association of Dermatologists Biologic Interventions Register were recruited into this UK, multi-centre observational cohort study (Biomarkers of Systemic Treatment Outcomes in Psoriasis). Serial samples were taken during adalimumab therapy and analysed for adalimumab level and ADAs. Response to treatment was defined as PASI75 for the primary outcome. Data from this cohort were used to determine: (i) the therapeutic range (n=303 patients; 409 samples) and; (ii) whether early drug levels predict response at 6 months (n=120 patients; 159 samples). Receiver Operating Characteristic analysis for PASI75 identified a minimally effective drug level of >3.2µg/ml (sensitivity: 80%, specificity: 58%, AUC: 0.74 (95%CI:0.68-0.79); response plateaus after a median drug level ≥4.6µg/L. We used mixed effect logistic regression to account for clustering of samples within patients as well as adjusting for co-variates (e.g. weight and presence of ADA).
This real-world study, with pragmatic drug level sampling indicates: (i) a therapeutic range for adalimumab with a minimum effective drug level and an upper bound of drug level which provides no additional therapeutic benefit; and (ii) evidence that early drug level monitoring can predict non-response and opportunity for treatment switch or dose escalation. These data support pro-active measurement of adalimumab drug levels to optimise therapy.

PSORT is MRC funded and has industry partners who have contributed funding for this work.

Keywords: psoriasis; adalimumab; therapeutic range; mixed models

P10

Juliane Mueller

Title: RNA analysis in patients with complex neurological diseases caused by mutations in the RNA exosome

Institute of Genetic Medicine (IGM)

The RNA exosome is a multiprotein complex essential for the degradation and processing of RNA in the nucleus and the cytoplasm. The exosome complex is well-conserved in all eukaryotic cells; its structure and function has mainly been studied in the budding yeast Saccharomyces cerevisiae. We and others have characterised patients suffering from complex inherited neurological diseases (pontocerebellar hypoplasia, spinal muscular atrophy) who carry autosomal recessive mutations in subunits of the exosome (EXOSC3, EXOSC8, EXOSC9), or the exosomal cofactor RBM7. Protein analysis in patient fibroblasts revealed a reduction of all exosome subunits in patient cells, indicating that the loss of one subunit reduces the total amount of available exosome complex in the cell. Given the relevance of the exosome complex for the cellular RNA metabolism, our aim was to investigate whether exosome subunit mutations lead to an abnormal RNA metabolism in patient cells. We performed RNASeq analysis in patient fibroblasts, muscle biopsy samples, and in neuronal progenitor cells obtained through reprogramming of fibroblasts. Initial results indicated changes in levels of mRNAs that contain AU-rich elements in their 3'-UTRs. Furthermore, we are planning to analyse changes in non-coding RNA levels and cryptic unstable transcripts (such as promoter upstream transcripts), as the exosome has been shown to be involved in their degradation. We hope that analysis of the RNA metabolism changes will help elucidate the disease mechanism of exosome related diseases.

Keywords: RNA exosome; inherited neurological disease; RNASeq

P11

Katie Thomson

Title: The effects of public health policies on health inequalities in welfare states: an umbrella review

Institute of Health and Society (IHS)

Background
Socio-economic inequalities are associated with unequal exposure to social, economic and environmental risk factors, which in turn contribute to health inequalities. Understanding the impact of specific public health policy interventions will help to establish causality in terms of the effects on health inequalities.

Methods
Systematic review methodology was used to identify systematic reviews from high-income countries that describe the health equity effects of upstream public health interventions. Twenty databases were searched from their start date until March 2016. The quality of the included articles was determined using the Assessment of Multiple Systematic Reviews tool (AMSTAR).

Results
Twenty-nine systematic reviews were identified reporting 150 unique relevant primary studies. The reviews summarised evidence of all types of primary and secondary prevention policies (fiscal, regulation, education, preventative treatment and screening) across seven public health domains (tobacco, alcohol, food and nutrition, reproductive health services, the control of infectious diseases, the environment and workplace regulations). There were no systematic reviews of interventions targeting mental health. Most of the reviews were of moderate quality, although the included primary studies were generally considered to be low in quality. Results were mixed across the public health domains; some policy interventions were shown to reduce health inequalities (e.g. food subsidy programmes, immunisations), others have no effect and some interventions appear to increase inequalities (e.g. 20 mph and low emission zones). The quality of the included reviews (and their primary studies) were generally poor and clear gaps in the evidence base have been highlighted.

Conclusions
The review does tentatively suggest interventions that policy makers might use to reduce health inequalities, although whether the programmes are transferable between welfare regimes is unclear.

Keywords: social determinants of health; equity; intervention

P12

Lauren Walker

Title: Does pyroglutamylated β amyloid associate with hyperphosphorylated tau in Alzheimer's disease?

Institute of Neuroscience (IoN)

Background
In Alzheimer's disease (AD) toxic proteins accumulate in the brain, namely β amyloid (forming plaques) and hyperphosphorylated tau (forming tau tangles). It is assumed that β amyloid interacts with hyperphosphorylated tau causing it to increase, which is linked to clinical symptoms associated with AD. However, there are several species of β amyloid and all may not interact with hyperphosphorylated tau. One peptide is pyroglutamylated-Aβ (pAβ) which has been shown to be more neurotoxic, resistant to proteases, and promotes self-aggregation compared to other Aβ peptides. Our aim is to identify if pAβ is associated with hyperphosphorylated tau in human brain tissue, and as tau tangles can be formed by phosphorylation at numerous sites on tau protein, determine at which site a putative interaction takes place.

Methods
Post-mortem tissue from the frontal and entorhinal cortices of 18 AD and 23 control patients were immunohistochemically stained for pAβ and tau at 2 separate phosphorylation sites (Ser202/205 and Ty18). Percentage area of immunopositivity was calculated using quantitative image analysis.

Results
All loads were significantly higher in AD as compared to controls ($p \leq 0.01$). However, regression analysis revealed that only frontal pAβ load independently predicted the presence of AD ($p=0.01$). In frontal and entorhinal cortices pAβ load independently predicted tau phosphorylated at both Ser202/205 and Ty18 ($p<0.001$).

Discussion
Here, we report an association between pAβ and tau in human brain tissue and an influence of frontal pAβ on the severity of clinical dementia in AD. Our findings further support the notion that pAβ may represent an important link between Aβ and tau and investigations into its role as a diagnostic and therapeutic target in AD are warranted.

Keywords: Alzheimer's disease; pyroglutamylated Aβ; Tau; pathology

P13

Sarah Charman

Title: A Novel Cardiac Output Response to Stress Test Developed to Improve Diagnosis of Heart Failure

Institute of Cellular Medicine (ICM)

Background: Primary care physicians lack access to an objective cardiac function test. This study for the first time describes a novel Cardiac Output Response to Stress (CORS) test developed to improve diagnosis and monitoring of heart failure in primary care, and investigates its reproducibility.
Methods and Results: Prospective observational study recruited 32 consecutive primary care patients (age, 63±9 years, female n=18). Cardiac output was measured continuously using the bioreactance method in supine and standing positions, and during two 3-min stages of a step-exercise protocol (10 and 15 steps per minute) using a 15-cm height bench. The CORS test was performed on two occasions i.e. Test 1 and Test 2. There was no significant difference between repeated measures of cardiac output and stroke volume at supine (6.2±1.4 vs. 6.3±1.7 L/min, p=0.84; 102±24 vs. 108±32 ml/beat, p=0.36), standing (5.7±2.1 vs. 5.7±1.9 L/min, p=0.99; 82±32 vs. 83±29 ml/beat, p=0.93), stage one- (8.5± 1.8 vs. 8.2±1.9 L/min, p=0.56; 104±26 vs. 104±27 ml/beat, p=0.99) and stage two- step-exercise (9.9±1.7 vs. 9.6±2.0 L/min, p=0.51; 109±29 vs. 111±26 ml/beat, p=0.76). There was a significant positive relationship between Test 1 and Test 2 cardiac outputs (r=0.92, p=0.01 with coefficient of variation of 7.1%). The mean difference in cardiac output (with upper and lower limits of agreement) between Test 1 and Test 2 was 0.1 (-1.9 to 2.1) L/min, combining supine, standing and step-exercise data.
Conclusions: The CORS, as a novel test for objective evaluation of cardiac function, demonstrates acceptable reproducibility and can potentially be implemented in primary care.

Keywords: heart failure; diagnosis; primary care; reproducibility

P14

Diana Papini

Title: A potential new error correction mechanism for chromosome segregation in anaphase

Institute of Cell and Molecular Biosciences (ICaMB)

The effective segregation of chromosomes is essential for genome integrity. Errors can lead to birth defects and may contribute to carcinogenesis.

To prevent mis-segregation, the sister kinetochores (Kts, large protein structure) must connect chromosomes to microtubules (MTs) from opposite spindle poles. Anaphase onset (chromosome separation) must be delayed until all chromosomes are correctly attached in a bi-oriented state.

Even in normal cells, numerous attachment errors occur upon bi-orientation. These errors are generally detected during prometaphase by surveillance mechanisms that allow their release and correction. To do this, the centromeric kinase Aurora-B is required.
However, some errors can persist and lead to the formation of lagging chromosomes (LCs) observed at the midzone during anaphase: potential source of aneuploidy.

To date, only formation of LCs has been well studied. It remains still unclear how the cell deals with LCs once they are formed, and whether they are subjected to any type of error correction after anaphase.

Aurora-B is the major regulator of error correction in early mitosis and destabilises chromosome attachments. At anaphase onset, Aurora-B transfers from chromosomes to central spindle, where sets up an activity gradient across the dividing cell, centred on the midzone. Because Aurora-B is removed from centromeres in anaphase, it is commonly assumed that its role in error correction ends at that point.
We hypothesize that Aurora-B gradient functions as “measuring ruler” allowing the cell to identify LCs and correct their Kt-MTs attachments after anaphase.
Our data shows that Aurora-B substrates involved in error correction during early mitosis are also phosphorylated on LCs in anaphase.
Therefore, we are investigating whether Aurora-B regulates chromosome attachments by kinetochore phosphorylation on LCs, and if this can control laggers fate and reduce the incidence of chromosome mis-segregation. These results could reveal a new error correction mechanism for chromosome segregation in anaphase.

Keywords: mitosis; chromosome segregation; lagging chromosomes; aneuploidy

P15

Daniel Erskine

Title: Vulnerability to Lewy body pathology

Institute of Neuroscience (IoN)

Introduction
Lewy body pathology has been described as developing in a stereotyped sequence suggesting it may spread in a manner reminiscent of that described previously for prion protein. However, spreading based purely on anatomical connectivity cannot be reconciled with the apparent preservation of some regions strongly interconnected with early predilection sites. Furthermore, even within regions vulnerable to Lewy body pathology, not all cell types are affected.
Methods
We obtained post-mortem human brain tissue from aged control cases without Lewy body pathology and cases with neocortical Lewy body disease. We quantified the expression of physiological α-synuclein across different brain regions and distinct cellular populations of control cases, and then compared this to the typical topography of Lewy body pathology.
Results
In neocortical Lewy body disease cases, Lewy body pathology shows a fairly consistent hierarchy of Lewy body pathological burden. In control cases, physiological α-synuclein has strikingly lower expression in brain regions that do not typically develop Lewy body pathology.
Discussion
We suggest these findings are consistent with prion-like nucleation and propagation, whereby the physiological protein must be induced to misfold by aggregated α-synuclein. Therefore, we speculate that lower normal levels of physiological α-synuclein may be prohibitive to the development of Lewy body pathology.

Keywords: Lewy; dementia; prion; alpha-synuclein

P16

Paul Sinclair

Title: Dynamic clonal progression in xenografts of acute lymphoblastic leukaemia with intrachromosomal amplification of chromosome 21.

Northern Institute for Cancer Research (NICR)

Intrachromosomal amplification of chromosome 21 is a heterogeneous chromosomal rearrangement occurring in 2% of childhood precursor B-cell acute lymphoblastic leukaemia. There are no cell lines with iAMP21 and these abnormalities are too complex to faithfully engineer in animal models. As a resource for future functional and pre-clinical studies, we have created xenografts from intrachromosomal amplification of chromosome 21 leukaemia patient blasts and characterised them by in-vivo and ex-vivo luminescent imaging, FLOW immunophenotyping, and histological and ultrastructural analysis of bone marrow and the central nervous system. Investigation of up to three generations of xenografts revealed phenotypic evolution, branching genomic architecture and, compared with other B-cell acute lymphoblastic leukaemia genetic subtypes, greater clonal diversity of leukaemia initiating cells. In support of intrachromosomal amplification of chromosome 21 as a primary genetic abnormality, it was always retained through generations of xenografts, although we also observed the first example of structural evolution of this rearrangement. Clonal segregation in xenografts revealed convergent evolution of different secondary genomic abnormalities implicating several known tumour suppressor genes and a region, containing the B-cell adaptor, PIK3AP1, and nuclear receptor co-repressor, LCOR, in the progression of B-ALL. Tracking of mutations in patients and derived xenografts provided evidence for co-operation between abnormalities activating the RAS pathway in B-ALL and for their aggressive clonal expansion in the xeno-environment. Bi-allelic loss of the CDKN2A/B locus was recurrently maintained or emergent in xenografts and also strongly selected as RNA sequencing demonstrated a complete absence of reads for genes associated with the deletions.

Keywords: leukaemia; PDX; clonal progression; morphology

P17

Ricardo Gouveia

Title: Brillouin spectro-microscopy as a new tool to explore stem cell niche biomechanics

Institute of Genetic Medicine (IGM)

Brillouin spectro-microscopy (BSM) is an innovative non-contact, non-destructive technique capable of characterising the mechanical properties of live cells and tissues with unprecedented sensitivity and resolution. In this study, we used BSM to investigate human corneal biomechanics, in particular within the corneal niche hosting the tissue's epithelial stem cell populations – the limbus. Our results revealed a correlation between the mechanical properties of the corneal matrix and the phenotype of corneal epithelial cells. Moreover, they confirmed that stiffness plays a fundamental role in directing the behaviour of corneal epithelial cells both within the limbus and across the corneal surface. Ultimately, this work demonstrated that BSM has matured to a level where it can now be extensively applied to several research fields, namely for studying mechanotransduction in human stem cell niches.

Keywords: Brillouin spectro-microscopy; biomechanics; stem cells; mechanotransduction

P18

Svetlana Cherlin

Title: Prediction of treatment response in Rheumatoid Arthritis patients using genome-wide SNP data

Institute of Genetic Medicine (IGM)

Although a number of treatments are available for Rheumatoid Arthritis (RA), each of them shows a significant non-response rate in patients. Therefore, predicting a priori the likelihood of treatment response would be of great benefit. Here we conducted a comparison of a variety of statistical methods for predicting the change in C-reactive protein score (CRP), in 28 swollen joint count score (SJC28) and in erythrocyte sedimentation rate (ESR) between baseline and 3 or 6 months using genome-wide SNP data from RA patients available from the MAximising Therapeutic Utility in Rheumatoid Arthritis (MATURA) consortium. Two different treatments (tumour necrosis factor α inhibitors and methotrexate) and nine different methods (lasso, ridge, elastic net, random forests, support vector regression, sparse partial least squares, genome-wide complex trait analysis, Bayesian sparse linear mixed model, and neural network) were evaluated. We used 10-fold cross validation to assess predictive performance, with nested 10-fold cross validation used to tune the model parameters when required. Overall, we found that SNPs add very little prediction information to that obtained using clinical information only, such as baseline trait value. This observation can be explained by the lack of strong genetic effects and the relatively small sample sizes available. However, methods that assume a complex underlying genetic architecture of the trait were able to extract some information about prediction, in comparison to methods that assume a simplified genetic architecture. Additionally, methods that are consistent with the genetic architecture of the trait were able to achieve better prediction than methods that are not.

Keywords: c ross-validation; prediction; SNP data; treatment response

P19

Oliver Shannon

Title: ASSOCIATION BETWEEN MEDITERRANEAN DIET ADHERENCE AND COGNITIVE FUNCTION IN A UK COHORT: THE EPIC-NORFOLK STUDY

Institute of Cellular Medicine (ICM)

The Mediterranean diet is characterised by a high intake of fruits, vegetables, olive oil, unrefined grains, legumes and fish, a moderate intake red wine, and a low intake of red meat. In Mediterranean countries, higher adherence to this dietary pattern has been associated with improved cognitive function and reduced dementia incidence (Galbete et al., 2015; Trichopoulou et al., 2015; Martinez-Lapiscina et al., 2013). However, the impact of a Mediterranean diet on cognitive function in a UK population is unknown. In this ongoing analysis, we are investigating associations between adherence to a Mediterranean diet and cognitive function using data from the European Prospective Investigation into Cancer and Nutrition, Norfolk (EPIC-Norfolk). Habitual diet of participants, measured using food frequency questionnaires at baseline (1993 – 1997; Health Check 1) and during follow up (1998 – 2000; Health Check 2), are being used to estimate Mediterranean diet adherence via the PREDIMED and Mediterranean Diet Pyramid scoring systems. We will examine the strength of associations between the composite diet scores and individual components of the Mediterranean diet, and cognitive function (including measures of global and domain specific function) measured at a second follow up (2004 – 2011; Health Check 3; n = 8623). In addition, we will explore i) the smallest effective change in Mediterranean diet score associated with measurable improvement in cognitive function, ii) the existence of a threshold effect above which further increases in Mediterranean diet score are not associated with cognitive benefits, and iii) the interaction between Mediterranean diet score, cognitive function, and apolipoprotein E (APOE) genotype and sex. Preliminary results from this analysis will be presented, alongside a discussion of the findings for the design of future randomised controlled trials. This analysis is supported by The Alzheimer's Research UK Prevention and Risk Reduction Fund (ARUK-PRRF2017-006).

Keywords: mediterranean diet; cognitive function; nutrition

P20

Martyna Pastok

Title: CDK4 and CDK6 regulators in the cell cycle and beyond.

Northern Institute for Cancer Research (NICR)

In normal cell, CDK4/6 in complex with cyclin D is required for G1 cell cycle progression. In cancer, disease of uncontrolled cell proliferation, CDKs and cyclins are often misregulated; therefore, they are attractive therapeutic target. The main focus of current inhibitor design is the ATP-binding pocket placed between two kinase lobes further called the ATP-competitive inhibitors, which currently belong to the biggest class of inhibitors used in clinic. Besides having a well-documented role in the cell cycle, CDK4, CDK6 and cyclin D have been implicated to have a role independent of cell cycle and are associated with transcription. CDK6 and cyclin D have been found on chromatin and a big genetic-proteomic study revealed that cyclin D1 occupies promoters of abundantly expressed genes. Additionally, CDK4 also has been found to exist in cytoplasm in high-molecular weight complexes: >450kDa and 200kDa, which would suggest it exists in big molecular assemblies. It is still not entirely understood if CDK4, CDK6 and cyclin D have specific transcriptional role or are rather part of large multiprotein scaffolds. I will present data looking at cyclin D transcriptional role using combination of cell-based, computational and biophysical assays. Further studies on structural and mechanistic level of CDK4(6)/cyclin D containing complexes and their role in non-cell cycle function is crucial to improve our understanding of function and regulation of various CDK complexes and can give important implications to better inhibitor design.

Keywords: CDK; cyclin; transcription

P21 (poster withdrawn)

Jonathan Richardson

Title: Pregnancy outcomes following maternal antihistamine use for atopy in pregnancy: A prospective observational cohort study

Institute of Cellular Medicine (ICM)

Background: Limited data are available concerning the safety of maternal antihistamine use for atopy in pregnancy. The aim of this study is to improve the currently limited evidence-base.
Methods: Pregnancy exposure and outcome information was collected by the UK Teratology Information Service (UKTIS) using standardised procedures. Pregnancies exposed to known or suspected human teratogens/fetotoxicants were excluded. The study sample consisted of pregnancies exposed to antihistamines in the treatment of maternal atopy and a matched (calendar year of pregnancy and maternal age) comparator group where maternal antihistamine use for any indication was not reported. Crude rates of adverse pregnancy outcomes were compared using exact methods. Binomial logistic regression and event history analysis techniques were used to adjust crude spontaneous abortion rates for relevant confounding factors.
Results: The study sample included 306 antihistamine-exposed pregnancies matched to 3060 antihistamine-unexposed controls. No statistically significant differences in crude rates of major congenital malformation (P-value 0.251), intrauterine death/stillbirth (P-value 1.00), small for gestational age (P-value 0.995) or preterm delivery (P-value0.475) were observed between the exposed and control groups. However, rates of spontaneous abortion among the antihistamine-exposed pregnancies were significantly increased after adjustment for variation in both maternal gestational age at reporting to UKTIS and rates of elective termination as a competing risk to spontaneous abortion (P-value 0.0433), and separately after adjustment for maternal demographic confounders (aOR 1.73, 95%CI; 1.12-2.62). Analysis of spontaneous abortion risks for specific antihistamines identified a statistically significant increased risk following maternal cetirizine use (aOR 3.13, 95%CI; 1.44-6.62), and non-significant increased risks for promethazine, fexofenadine and lortadine.
Discussion: Chance, methodological limitations and/or data confounding may explain these findings, however, given the widespread use of antihistamines in pregnancy, further investigations to replicate or refute these findings using alternative datasets is recommended.

Keywords: antihistamines; atopy; pregnancy; miscarriage; congenital malformation

P22

Richard Howey

Title: Imputation of Missing Data for Bayesian Network Analyses of Complex Biological Data

Institute of Genetic Medicine (IGM)

Bayesian networks have been proposed as a way to identify possible causal relationships between measured variables based on their conditional dependencies and independencies, particularly in complex scenarios with many variables. When there is missing data, the standard approach is to remove individuals with missing data before performing Bayesian network analyses. This is undesirable when there are many individuals with missing data, perhaps with only one variable missing. Thus, imputation of the missing data is a natural choice. We present a new imputation approach designed to increase the power to detect causal relationships whilst accounting for model uncertainty. This method uses a version of nearest neighbour imputation, whereby missing data from one individual is replaced with data from another individual, their nearest neighbour. An important feature of this approach is that it can be used with both discrete and continuous data. For each individual with missing data, a single bootstrap iteration of the complete data is used to estimate a preliminary Bayesian network. Subsets of variables that have close connections to the missing variables are then chosen to find the nearest neighbour. We show that use of our imputation method increases the power to detect the correct model in simulated data by as much as over 50%. Such increases may be possible in real data when most individuals have missing data due to cost or practical reasons. Thus, the use of our imputation method has great potential to boost the power of Bayesian network analyses to identify possible causal relationships.

Keywords: causal; Bayesian network; imputation; missing data

P23

Monika Olahova

Title: CRISPR/Cas9-mediated knockout of RTN4IP1 leads to a severe mitochondrial Complex I assembly defect.

Institute of Neuroscience (IoN)

Mitochondrial disorders are a highly heterogeneous group of diseases caused by impaired mitochondrial function. Optic neuropathies are neurodegenerative disorders frequently caused by variants in nuclear-encoded mitochondrial genes. Whole-exome sequencing identified RTN4IP1, encoding a Reticulon 4 Interacting Protein 1, as a disease gene causing isolated optic atrophy, lactic acidosis, hypotonia and early-onset encephalopathy 1. Patient cells harbouring compound heterozygous pathogenic variants in RTN4IP1 showed a complete loss of RTN4IP1 protein and a mitochondrial respiratory chain Complex I (CI) assembly defect 1. To further investigate the molecular basis of the newly identified mitochondrial disease-causing gene RTN4IP1 using a CRISPR/Cas9-mediated RTN4IP1 knockout human cell line model. CRISPR/Cas9-mediated knockout of RTN4IP1 resulted in a generation of a human cell line carrying biallelic RTN4IP1 variants. The mutant RTN4IP1 protein was completely absent in the RTN4IP1 knockout cells. Western blotting, blue-native PAGE, complexome profiling analysis and high-resolution respirometry was used to assess the role of RTN4IP1 in mitochondrial function. Characterisation of CRISPR/Cas9-mediated RTN4IP1 knockout cells showed a marked decrease in the steady-state levels of the mature CI and its subunits. These data recapitulate the CI defect observed in the RTN4IP1 patient fibroblasts. Moreover, high-resolution complexome profiling of the RTN4IP1 knockout cells revealed an abnormal assembly of the CI N-module and PND5 module as well as a complete absence of the FOXRED1 assembly factor in CI intermediates. In addition, we also detected a marked reduction in the mitochondrial respiratory chain supercomplex. Our data suggest that RTN4IP1 encodes a novel assembly factor that has an essential role in the late stage of mitochondrial CI assembly pathway and demonstrates the utility of CRISPR/Cas9-mediated knockouts to model pathogenic phenotypes with potential to investigate the molecular function.

1 Charif M et al. Neurologic Phenotypes Associated with Mutations in RTN4IP1 (OPA10) in Children and Young Adults. JAMA Neurol. 2017

Keywords: mitochondrial disease; Complex I; RTN4IP1; CRISPR/Cas9

P24

Vinciane Saint-Criq

Title: Identifying new therapeutic targets for Cystic Fibrosis airway disease

Institute of Cell and Molecular Biosciences (ICaMB)

Cystic Fibrosis (CF) is the most common genetic condition in the UK and is caused by mutations in the CFTR gene which compromises salt and fluid transport across epithelia. Although CF affects many organs, morbidity and mortality is dominated by progressive lung destruction due to a build-up of thick and sticky mucus. The airway epithelium is covered by an aqueous layer (Airway Surface Liquid, ASL), which is dehydrated and acidic in CF and provides a favourable environment for bacterial growth. The acidic pH worsens mucus stickiness and prevents the removal of pathogens trapped in the mucus. These hallmarks of CF lung disease are also found in other obstructive lung pathologies such as asthma or COPD.

Although recently there has been remarkable advances in treatments for CF, targeting the basic CFTR permeability and trafficking defects, clinical benefit often depends on the CFTR mutation(s) and even within the same genotype, responses largely differ from one individual to another. There is therefore an unmet need for new drugs that will bypass CFTR, restore an efficient mucociliary clearance independently of the genotype and which could also be used to treat other obstructive lung diseases.

Our approach is to target the acid-base homeostasis in order to restore ASL pH, hydration and mucus clearance and therefore improve the lung pathophysiology of individuals with these diseases. We have developed a new method combining a cell-impermeant pH-sensitive dye and atmosphere-controlled plate-reader that enables dynamic measurements of ASL pH. Our technique delivers stable measurements on primary cells under near in vivo conditions and has helped us identify (i) two new ion transporters involved in ASL pH regulation and (ii) a commercially available small molecule that restores ASL pH, hydration and mucus transport in CF cells. These could serve as potential therapeutic targets in obstructive lung diseases.

Keywords: cystic fibrosis; pH; epithelium; ion channels/transporters

P25

Ella Dennis

Title: THE ROLE OF A NOVEL PROTEIN FOLDING CHAPERONE, CRELD2, IN SKELETAL DEVELOPMENT AND DISEASE

Institute of Genetic Medicine (IGM)

Cysteine rich with epidermal growth factor domains 2 (CRELD2) has previously been identified as a novel endoplasmic reticulum (ER) stress-inducible protein disulphide isomerase (PDI) implicated in the pathogenesis of skeletal dysplasias. The function of CRELD2 is largely unknown despite putative roles described in protein folding and trafficking. Creld2 is expressed in mouse embryonic skeletal tissues and interestingly a novel role has been identified for CRELD2 in mesenchymal stem cell osteogenic differentiation. To further study the role of Creld2 in skeletal development and disease, conditional Creld2 knockout mice were generated and phenotyped.

We have shown that cartilage-specific Creld2 knockout mice display a distinctive chondrodysplasia phenotype characterised by disproportionate short statue and a disrupted cartilage growth plate. For example, the ablation of Creld2 in the growth plate significantly reduces chondrocyte proliferation and survival resulting in regions of hypocellularity and a striking misalignment of chondrocytes within individual chondrons and columns. Interestingly, the organisation of chondrocyte primary cilia is impaired indicating a loss of chondrocyte polarity, which could also impair the columnar organisation of growth plate chondrocytes.

We have also generated and phenotyped bone-specific Creld2 knockout mice. These mice display an osteopenic phenotype characterised by a reduction in bone mass with altered trabecular architecture. The deletion of Creld2 in osteoblasts significantly impairs osteoblast survival and interestingly induces the upregulation of osteoblast-derived osteoclastogenic cytokine production, disrupting the balance between bone formation and resorption.

The work presented here shows that CRELD2 plays distinct roles in bone and cartilage and points to an important role for this novel protein-folding chaperone in chondrocyte differentiation, osteoblast function and skeletal development.

Keywords: cartilage; bone; genetics; rare disease

P26

Onur Sen

Title: The live cell DNA stain SiR-Hoechst induces DNA damage responses and cell cycle arrest

Institute of Cell and Molecular Biosciences (ICaMB)

SiR-Hoechst (SiR-DNA) is a far-red fluorescent DNA probe being used widely for time-lapse imaging of living cells that is reported to be minimally toxic at concentrations as high as 10 - 25 µM. However, measuring nuclear import of Cyclin B1, inhibition of mitotic entry, and the induction of gamma-H2AX foci in cultured human cells reveals that SiR-Hoechst induces DNA damage responses and G2 arrest at 1 µM concentration and below. SiR-Hoechst is useful for live cell imaging, but it should be used with caution and at the lowest practicable concentration.

Keywords: live cell imaging; DNA damage

P27

Helen Griffin

Title: A Multiomic Approach to Identify Genetic Modifiers of Reversible Infantile Respiratory Complex Disorder

Institute of Genetic Medicine (IGM)

Reversible Infantile Respiratory Complex Disorder (RIRCD) is a mitochondrial myopathy primarily caused by the homoplasmic m.14674T>C mt-tRNAGlu mutation which shows incomplete penetrance and unlike other mitochondrial disorders, affected patients with severe muscle weakness have the unusual characteristic of spontaneous recovery from 5 months of age. We are working to identify other genetic modifiers of RIRCD using a multiomic approach of exome sequencing, RNA sequencing and proteomic profiling. Exome sequences were obtained from 21 affected and 12 unaffected individuals from 14 families that carry the mtDNA mutation. RNA sequencing was performed on skin fibroblasts and skeletal muscle biopsies from affected, unaffected and recovered individuals, and also non-RIRCD mutation carriers. Proteomic profiling was performed on skeletal muscle biopsies from affected and recovered patients, and control individuals. RNA sequencing identified 96 genes in fibroblasts and 786 genes in muscle that showed >2-fold change in expression between affected and unaffected mutation carriers. The mitochondrial translational activator (MSS51) showed >10-fold reduction in expression in affected versus unaffected muscle. Proteomic profiling identified >2-fold differences in abundance for 485 out of 1672 proteins. Biological pathways identified by gene set enrichment analysis of differentially expressed genes and proteins included those involved with mitochondrial energy metabolism, RNA translation and muscle contraction. Exome sequences included conserved and damaging variants in genes belonging to relevant biological pathways. It is hoped that by identifying genetic modifiers of RIRCD and understanding the mechanisms behind the recovery process, we will uncover genetic variants and pathways that are also important in the aetiology of other more severe mitochondrial disorders.

Keywords: bioinformatics; mitochondrial DNA

P28

Michael Ortiz Rios

Title: Refined methods for acrylic-free MRI-compatible implants in nonhuman primates for neuroimaging and electrophysiology

Institute of Neuroscience (IoN)

Nonhuman primate neuroscientists commonly use permanently implanted head posts to stabilize the head during electrophysiology or functional magnetic resonance imaging (fMRI) data acquisition. Additionally, recording chambers are implanted for chronic neurophysiological and/or interventional approaches. To ensure MRI compatibility, implants are typically made out of thermoplastic material or polytheretherketone (PEEK) and fixed onto the skull using layers of acrylic or bone cement around the implant. Here, we report on a refined method for MRI-compatible implants made out of PEEK, which are shaped to the individual animal skull secured with ceramic screws, without acrylic or bone cement for stabilization. For custom design, a 3D reconstruction of the individual animal skull is created based on a T1-MRI scan and a computer aided design (CAD) file that is placed over the region of interest. A 5-axis computer numerical control (CNC) machine is used to create the implant based on the intersection with the bone. After manufacturing, the base of the implant is coated with hydroxyapatite (HA) to promote osseointegration. To date, implants on four animals fit precisely onto the skull, integrate with bone and remain stable for more than a year. We prevent infections of the wound margin by using subcuticular suture techniques and a protective cap worn after recovery. Overall, our custom PEEK design with osteocompatible properties, facilitates surgical procedures, induces bone integration, ensures long-term stability and preserves a healthy skin margin without the need of regular cleaning. Overall, our refined methods allow us to obtain distortion-free fMRI data without signal drop-out and higher signal-to-noise ratio during awake imaging as compared to animals with cement implants. We believe that our methods, in particular the long-term stability of the PEEK implants, might further contribute to the wellbeing of nonhuman primates in neuroscience research.

Keywords: fMRI; PEEK; HA; macaque; implants

P29

Jad Sassine

Title: The absence of LTA glucolipids impacts cell wall synthesis in Bacillus subtilis

Institute of Cell and Molecular Biosciences (ICaMB)

The peptidoglycan (PG) layer is responsible for maintaining cell shape and permitting cell division in almost all bacteria. Made of glycan chains connected by short peptides, PG forms a net-like structure surrounding the cytoplasmic membrane. Cell wall growth is facilitated by PG synthases and hydrolases. Genetic and phenotypic analysis of B. subtilis strains has shown that PG synthesis and cell division are modulated by components of the central carbon metabolism. In particular, UgtP, which synthesises the glucolipid precursor for lipoteichoic acid, has been suggested to function as a metabolic sensor governing cell size. However, the mechanism by which UgtP impacts cell wall synthesis remained unknown. The PG composition of ugtP mutant cells suggested an increased DL-endopeptidase activity. The double deletion of ugtP and lytE, encoding a hydrolase important for cell elongation, produced short and bent cells with severe shape defects. Interestingly, the ugtP lytE mutant recovered rod-shape by mutations that decreased the expression of the PG synthase PBP1. Metabolomics analysis for the ugtP mutant showed increased levels of cell wall precursors. These results suggest that cells react to the loss of UgtP by modifying PG synthesis and hydrolysis, and that balancing PG synthesis and hydrolysis by PBP1 and LytE is crucial for the ugtP mutant to maintain rod-shape. These results suggest that cells react to the loss of UgtP by balancing PG synthesis and hydrolysis by PBP1 and LytE, respectively.

Keywords: peptidoglycan; synthesis; hydrolysis; LTA; metabolism

P30

Gerrit Hilgen

Title: Pan-retinal characterisation of parvalbumin-expressing cells in the mouse retina

Institute of Neuroscience (IoN)

Using high resolution microscopy, we have investigated the immunohistochemical identity of PV-expressing cells at pan-retinal level in mouse by looking at co-expression of PV and various RGC and AC markers, including calretinin, an additional Ca2+ binding protein. In another series of experiments, we have investigated the functional properties of PV-expressing cells across the retina using large scale electrophysiology recordings with a high-density large-scale Multielectrode Array (Biocam, 3Brain) combined with pharmacogenetics. We used a mouse model in which PV+ cells express silencing Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). In the presence of the DREADD agonist clozapine N-oxide (CNO), the DREADD-PV cells hyperpolarize, resulting in substantial changes in firing rate. Various stimuli were used to characterize RGC receptive field organization, including motion and orientation sensitivity in control conditions and during PV cell silencing.

We found that PV RGCs are overall evenly distributed across the retina. Peripheral PV+ cells exhibited more co-expression with calretinin, a novel finding in the retina, so far only reported in cortical cells. At this point we have not yet been able to determine whether these cells tend to be RGCs or rather ACs, or both.
PV+ RGCs were identified according to their decreasing neural activity in the presence of CNO. Overall, we found a high incidence to OFF responses amongst PV-RGCs, whereas non-PV RGCs tend to have a higher incidence of ON responses. Both ON and OFF responses in PV-RGCs became more prolonged, blurring the distinction between transient and sustained responses. Finally, we found that oscillations induced in RGCs during specific visual tasks became much stronger in the presence of CNO, presumably because of silencing of PV+ ACs. Developing a more sophisticated method for cell functional and anatomical classification will help us decipher pan-retinal topographic variability in the expression of specific subclasses of PV-expressing RGCs.

Keywords: retina; vision; sensory systems; retinal circuitry

P31

Alison Wheatley

Title: Improving care for falls in dementia: development of the DIFRID intervention

Institute of Health and Society (IHS)

Background: People with dementia living in their own home experience ten times as many falls as people without dementia. Little is known about how best to deliver services to people with dementia following a fall. This study aimed to develop an intervention to improve care for this patient group following a fall requiring healthcare attention.
Methods: We used an integrated, mixed-methods approach to intervention development which combined theory generated via a realist synthesis and data on current provision and pathways gathered through a prospective observational study as well as qualitative interviews, focus groups, and ethnographic observation. A modified Delphi panel approach with a group of 24 experts was used to translate theory into priorities for a new intervention.
Results: Consensus was achieved on 52 statements over two rounds of surveys. The statements were compiled and used to model the proposed intervention, which is a complex multidisciplinary intervention taking place mainly in the patient's home over a period of 12 weeks.
Conclusion: This intervention has the potential to improve outcomes of falls in people with dementia. It is now being tested in a feasibility study. This paper describes the intervention development process, including theory generation and consensus-seeking.

Keywords: dementia; falls; intervention development; qualitative; realist

P32

Iglika Ivanova

Title: PERK/eIF2α signaling inhibits HIF-induced gene expression during the UPR via YB1 regulation of HIF1α translation

Institute of Cell and Molecular Biosciences (ICaMB)

HIF1α (hypoxia inducible factor 1α) is the central regulator of the cellular response to low oxygen and its activity is deregulated in multiple human pathologies. Consequently, given the importance of HIF signaling in disease, there is considerable interest in developing strategies to modulate HIF1α activity and down-stream signaling events. In the present study we find that under hypoxic conditions, activation of the PERK branch of the unfolded protein response (UPR) can suppress the levels and activity of HIF1α by preventing efficient HIF1α translation. Activation of PERK inhibits de novo HIF1α protein synthesis by preventing the RNA-binding protein, YB-1, from interacting with the HIF1α mRNA 5'UTR. Our data indicate that activation of the UPR can sensitise tumor cells to hypoxic stress, indicating that chemical activation of the UPR could be a strategy to target hypoxic malignant cancer cells.

https://doi.org/10.1093/nar/gky127

Keywords: translation; hypoxia; HIF1a; Yb1 signalling

P33

Lindi Chen

Title: Preclinical assessment of MDM2, ALK and MEK inhibitor combinations in neuroblastoma

Northern Institute for Cancer Research (NICR)

Background: Treatment of patients with high-risk neuroblastoma remains clinically challenging. The use of novel small molecule inhibitors targeting oncogenic pathways perturbed in neuroblastoma offers a non-DNA damaging, potentially more effective and less toxic treatment strategy. A high proportion of aberrations upstream of p53, and an increased incidence of ALK and RAS/MAPK pathway abnormalities have been observed in relapsed neuroblastoma, which support the use of MDM2, ALK and MEK inhibitors as potential novel therapies in high-risk neuroblastoma.
Methods and Results: Using XTT assays, GI50 values of multiple ALK and MEK inhibitors were determined in a large panel of neuroblastoma cell lines of varying ALK and MAPK pathway status. Sensitivity to MEK inhibitors correlated with the presence of a MAPK pathway aberration with cell lines with RAF/RAS aberrations being more sensitive than those with NF1 aberrations. Sensitivity to ALK inhibitors, correlated with the presence of an ALK aberration, with cell lines with ALK mutations being more sensitive than those with amplification or copy number gain. Using median-effect analysis, selected 2-way combinations of idasanutlin, ALK and MEK inhibitors were shown to be synergistic in cell lines with wild-type p53 and an ALK or MAPK aberration. Consistent with this, in most cases, combination treatments led to increased levels of apoptosis as evident by higher levels of caspase 3/7 activity, compared to either agent alone.
Conclusions: These data highlight differences between the tested ALK inhibitors and type of ALK aberration with ALK amplified cell lines being less sensitive despite the potential clinical significance of ALK amplification in high-risk neuroblastoma. The data also show that combinations of MDM2, ALK and/or MEK inhibitors are a potential therapeutic option for neuroblastoma patients with functional p53 and aberrations in ALK and/or the MAPK pathway and should be further explored in in vivo models.

Keywords: neuroblastoma; ALK; MDM2; MAPK; combination therapy

P34

Marta Markiewicz-Potoczny

Title: A critical role for Dna2 at unwound telomeres

Institute of Cell and Molecular Biosciences (ICaMB)

We produce evidence that Dna2, a conserved nuclease and helicase, plays a critical function at yeast telomeres. Dna2 functions redundantly with other proteins in Okazaki fragment processing, double strand break (DSB) resection and checkpoint kinase activation. Dna2 is an essential enzyme, required for yeast and mammalian cell viability. We report that numerous mutations affecting the DNA damage checkpoint suppress dna2Δ lethality in Saccharomyces cerevisiae. dna2Δ cells are also suppressed by deletion of helicases, PIF1 and MPH1, and by deletion of POL32, a subunit of DNA polymerase δ. All dna2Δ cells are temperature sensitive, have telomere length defects, and low levels of telomeric 3' single stranded DNA (ssDNA). Interestingly, Rfa1, a subunit of the major ssDNA binding protein RPA, and the telomere specific ssDNA binding protein Cdc13, often co-localize in dna2Δ cells. This suggests that telomeric defects often occur in dna2Δ cells. There are several plausible explanations for why the most critical function of Dna2 is at telomeres. Telomeres modulate the DNA damage response (DDR) at chromosome ends, inhibiting resection, ligation and cell cycle arrest. We suggest that Dna2 nuclease activity contributes to modulating the DNA damage response at telomeres by removing telomeric C-rich 5’ ssDNA and thus preventing checkpoint activation. This activity may be conserved in all eukaryotes.

Keywords: Dna2; telomere; yeast; genetics; DNA damage

P35

Fei Gao

Title: Signalling pathway involved with monitoring mitochondrial ribosomal stress

Institute of Neuroscience (IoN)

Mitochondria are well known as the ‘power house’ of cells. Defective mitochondria cause many forms of human disease affecting almost every tissue in the body and various studies have been performed to discover the genes responsible. ERAL1 (Era like 12S mitochondrial rRNA chaperone 1) localizes to the mitochondrion and is encoded by nuclear gene Eral1. Loss of ERAL1 does not affect mtDNA level, 39S-mt-LSU, oxidative phosphorylation, mitochondrial mass or ROS generation. However, depletion of ERAL1 decreases 12S rRNA level and causes cell growth arrest. The mechanism responsible for this signalling is still unknown. We applied whole-genome gene expression analysis, metabolomics study and kinome assay to find the related pathway causing cell growth arrest following ERAL1 depletion. Our studies found that after ERAL1 is depleted in U20S cells, mitostress will active PI3K pathway which connects with NFkB pathway. Activated RelA, component of NFkB complex, will promote p21 (CDKN1A) transcription and further inhibit cell cycle progress. Depleting RelA and some members of PI3K pathway could let cells lacking ERAL1 re-entry the cell cycle.

Keywords: mitochondria; signalling pathway

P36

Jill Hunter

Title: Regulation of checkpoint kinase signaling and tumourigenesis by the NF-kB regulated gene, CLSPN

Institute of Cell and Molecular Biosciences (ICaMB)

Aberrant NF-κB activity is associated with multiple aspects of cancer cell biology. Here we demonstrate the importance of this pathway as a regulator of the Chk1 checkpoint kinase in vivo. Chk1 has previously been shown to phosphorylate the RelA(p65) NF-κB subunit on Thr505 following DNA replication stress, resulting in inhibition of RelA pro-proliferative and anti-apoptotic functions. However, until recently, these studies were limited to in vitro analysis. Here, using both RelA T505A knockin and c-Rel knockout mice, we observed loss of Claspin expression in the Eμ-Myc B-cell lymphoma model. Claspin is a regulator of Chk1 activity and consistent with this observation these NF-κB mutant cells displayed reduced Chk1 activation. Significantly, Claspin levels correlated with survival, with both NF-κB mouse models as well as wild type mice with low Claspin levels displaying earlier onset of lymphoma. Interestingly, both RelA T505A and c-Rel null lymphomas were resistant to Chk1 inhibition, highlighting a potential route of chemo-resistance to the clinical use of Chk1 inhibitors for haematological malignancies. CLSPN heterozygous animals developed normally but exhibited aberrant hepatocyte proliferation following liver partial hepatectomy and increased liver injury in response to acute exposure with the DNA-damaging agent N-nitrosodiethylamine (DEN), suggesting an underlying genomic instability in these animals. Moreover, CLSPN heterozygous mice exhibited earlier onset of cancer in the DEN model of hepatocellular carcinoma, and an aged colony of unchallenged animals spontaneously developed lymphoid tumours. Taken together, these data demonstrate the significance of NF-κB regulation of Claspin and checkpoint kinase signalling in genomic instability and cancer onset.

Keywords: NF-κB, Claspin, Chk1, lymphoma, Chk1 inhibitor

P37 (poster withdrawn)

Tengfei Wan

Title: Boosting telomerase improves motor function and brain alpha-synuclein degradation in a Parkinson's disease mouse model

Institute of Cell and Molecular Biosciences (ICaMB)

While telomerase maintains telomeres in dividing cells, its protein component telomerase reverse transcriptase (TERT) has various non-canonical functions, such as localisation to mitochondria resulting in decreased oxidative stress, apoptosis and DNA damage. TERT protein is maintained in adult human and rodent brains predominantly in neurons, independently of telomerase activity which is downregulated early during development. We recently demonstrated increased mitochondrial TERT in hippocampal neurons from Alzheimer's disease brains and a beneficial function for TERT protein decreasing mitochondrial oxidative stress and lipid oxidation in tau transduced neurons in vitro (Spilsbury et al., Journal of Neuroscience, 2015). Since mitochondrial dysfunction is also involved in the development of Parkinson's disease (PD), we analysed the effects of boosting TERT levels by two telomerase activators on mitochondrial and motor function such as mitochondrial reactive oxidative species (ROS) levels, balance and gait, as well as on brain pathology in a transgenic PD mouse model over-expressing human α-synuclein.
TERT expression was increased in the brain tissue from transgenic mice after oral treatment with the activators for 14 months. This correlated well with the improvement of motor- and non-motor functions in treated mice. Moreover, mitochondrial ROS release, α-synuclein amount, phosphorylation and aggregation in the brain tissue were decreased after the treatment. Since the TERT protein has recently been shown to improve proteasomal and autophagy degradation, we hypothesis that this mechanism could be responsible for the lower α-synuclein levels.
We conclude that treatment with telomerase activators was able to ameliorate several PD-related symptoms in a mouse model over-expressing α-synuclein. Thus, our results suggest that boosting TERT levels might form a novel therapeutic treatment option for neurodegenerative diseases such as PD.

Keywords: Parkinson's, telomerase, alpha-synuclein, neurodegeneration, mitochondria, therapy

P38 (poster withdrawn)

Anthony Lagnado

Title: Neutrophils accelerate telomere-dependent senescence *in vitro* and *in vivo*

Institute of Cell and Molecular Biosciences (ICaMB)

Senescence, the state of irreversible arrest observed in somatic cells is characterised by a Senescent Associated Secretory Phenotype (SASP) which includes pro-inflammatory cytokines, chemokines and extracellular matrix proteases. The SASP is believed to play a role in the recruitment and activation of immune cells, including macrophages, CD4 T and NK cells which have been shown to play a role in clearance of senescent cells. However, the relationship between neutrophil recruitment and senescence has not been completely investigated.
We show that co-culture between young human fibroblasts and young neutrophils for 3 days leads to a significant reduction in the replicative lifespan of fibroblasts. Human fibroblasts pre-cultured with neutrophils experienced accelerated telomere shortening and increased expression of a variety of senescent markers. Pre-treatment with the enzyme catalase or ectopic overexpression of telomerase prevented the effects of neutrophils on senescence of human fibroblasts, suggesting a role for oxidative stress-mediated telomere shortening in the process.
Consistent with a role for neutrophils in telomere dependent senescence, we found an association between neutrophil infiltrations and telomere dysfunction in ageing mice.
Furthermore, we showed that induction of liver injury induced by CCl4 ,which resulted in increased neutrophil infiltrations contributed to telomere dysfunction in hepatocytes. Consistent with a role for neutrophils in the process, inhibition of neutrophil recruitment using the neutralising antibody Ly6G or in mice lacking TLR2 prevented CCl4 induced telomere dysfunction.
Our results suggest that neutrophils as a consequence of their role in the immune system may inadvertently induce senescence in young cells via oxidative stress-mediated telomere dysfunction.

Keywords: senescence neutrophils ageing telomeres ROS oxidation

P39

Anastasia Hepburn

Title: Induction of pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance.

Northern Institute for Cancer Research (NICR)

Emerging and established data shows importance of stem cell biology in cancer. Across many cancers, embryonic stem cell related gene expressions appear to confer an adverse prognosis. However more recently we have acquired an understanding of core master regulators of stem cell signatures that are limited to a small cohort of pluripotent inducing transcription factors – OCT4, SOX2 and NANOG (OSN). What is unknown is if these pluripotent factors act as master regulators of cancer stem cell biology and define a suitable target for treatment. In this work, we developed a preclinical in vitro stem cell culture model and show OSN are upregulated in a number of human epithelial cancers defining poor prognosis and treatment resistance. Significantly, overlapping pathways are detected and a common target is identified, DIO2. In a detailed case study of advanced prostate cancer we show DIO2 is a key actionable target. We therefore highlight the translational opportunity of manipulating stem cell regulators to tackle treatment resistance in cancer.

Keywords: cancer, pluripotency master regulators, treatment resistance

P40

Simon Tual-Chalot

Title: Endothelial endoglin is required to protect against high output heart failure.

Institute of Genetic Medicine (IGM)

Objectives: Endoglin is a co-receptor for TGFbeta/BMP9/10 signalling and ENG mutations lead to the vascular disorder hereditary haemorrhagic telangiectasia type I (HHT). Endoglin is also required for normal vascular development and angiogenesis, but little is known about endoglin's role in quiescent adult vascular endothelium.
Methods: To investigate this role, tamoxifen was administered to adult Cdh5(PAC)-CreERT2;Engfl/fl mice to generate endothelial-specific depletion of endoglin (Eng-iKOe). Cardiac magnetic resonance imaging, myography, vascular casting, microsphere injection, immunohistology, qPCR and aortic telemetry were used to evaluate cardiovascular changes after endoglin knockdown.
Results: Endothelial-specific loss of endoglin leads to an enlarged heart and cardiomyocyte hypertrophy within 5 weeks, progressing to high output heart failure (HOHF). In vivo aortic telemetry revealed significant loss of aortic pressure within a few days of endoglin depletion. Increased cardiac size and reduced cardiac afterload were confirmed by ventricular pressure loop analysis. As HOHF could result from arteriovenous malformations (AVMs), and these are found primarily in mucocutaneous and pulmonary tissues in HHT, we systematically screened for AVMs using microspheres and vascular casting. Although AVMs were absent in the majority of tissues, they were observed in the pelvic region and may account for the rapid increase in cardiac output. Having also observed an increase of VEGF-A protein in tissues from Eng-iKOe mice, we found that inhibition of VEGFR2 was protective against enlargement of the heart and dilatation of the ventricles.

Conclusion: Our results showed the essential role of endoglin in the maintenance of adult cardiovasculature through crosstalk with the VEGF signalling pathway.

Keywords: cardiac function endoglin

P41

Alem Gabriel

Title: Relapse specific genomic alterations in UK neuroblastomas detected by exome- sequencing and SNP-arrays

Northern Institute for Cancer Research (NICR)

Background: Neuroblastoma is the most common extracranial solid tumour in children and accounts for 8% of all childhood cancers in the UK. Relapsed neuroblastoma in particular remains a major challenge. Identification of new genetic abnormalities at relapse is needed to predict response to existing targeted agents as well as identify potential new treatment targets.
Aims: To study paired neuroblastoma tumours at diagnosis and relapse to determine the frequency of relapse specific mutations and new copy number abnormalities.
Methods: 38 paired neuroblastoma DNAs from diagnosis and relapse (16 high, 10 intermediate and 12 low risk) were studied, 35 by whole exome sequencing (WES) (Truseq Rapid Exome Kit) to a read depth of 100x including 5 germline DNAs at 30x to subtract variants from matched diagnostic DNAs. 18 pairs were run on SNP arrays (Illumina CytoSNP-850k chip) and analysed using Nexus 8 software. 15 pairs had matched data from SNP arrays and WES.
Results: WES revealed an average of 47 relapse specific missense and stop-gain variants (range 11-176) including ALK in 3 cases. Recurrent variants in ≥ 5 cases in MUC4, MUC17, MUC22, IGFN1 and KMT2C were observed. Germline exome data altered <5% of calls. In addition, 3 cases with a mutator phenotype were identified. Two high-risk cases had late relapses spanning 18 years and 10 years respectively. 384 copy number abnormalities (CNAs) were detected in 18 pairs with an increase in CNAs at relapse). Chromothripsis was observed in 2 cases. MDM2 amplification was detected (at diagnosis and relapse) in 1 case and an intragenic deletion of ATRX at relapse in another.
Conclusions: Mucin genes and pathways may provide new targets for drug development in neuroblastoma. Relapse cases with a mutator phenotype were detected in 9% of cases. WES of paired samples can be accurately analysed in the absence of germline data.

Keywords: Neuroblastoma, Cancer, relapse, WES, SNP-array

P42

Laura Ridgley

Title: IL-6 mediated programming of naive CD4+ T-cells in early rheumatoid arthritis drives dysregulated effector function

Institute of Cellular Medicine (ICM)

Evidence that IL-6 mediated activation of signal transduction and activator of transcription-3 (STAT-3) signalling amongst circulating CD4+ T-cells in early rheumatoid arthritis (RA) patients represents a relevant mechanism of disease induction was sought.

Paired serum and fresh whole blood from treatment-naïve early arthritis patients underwent IL-6 immuno-assay and flow cytometric phenotyping, respectively. In parallel, an in vitro model of naïve CD4+ T cell exposure to chronic, pathological concentrations of IL-6 was established. The dynamic transcriptional and functional consequences for downstream effector cells were then determined by microarray profiling and flow cytometry.

Maximal pSTAT3 levels, correlating most strongly with paired serum IL-6 concentrations, are observed in naïve CD4+ T-cells of early arthritis patients compared to their antigen experienced counterparts. Sensitivity to IL-6 seemed to reflect gp130 expression, which, unlike IL-6R, is also expressed maximally by the naïve cell subset. Pre-exposure of healthy control naïve CD4+ T-cells to pathological levels of the cytokine caused induction of STAT3 target genes known to discriminate RA patients from disease controls in the clinic. After TCR stimulation IL-6 pre-exposed cells exhibited enhanced proliferative capacity, activation and a propensity towards Th1 differentiation, compared to non-exposed cells. An analogous phenotype was observed in early RA compared to healthy donor CD4+ T-cells, with increased ki67 and CD25 expression, and increased IFN-γ production following stimulation.

IL-6 may “prime” the adaptive immune system to respond aberrantly to TCR stimulation in a manner that is not necessarily antigen-specific, potentiating RA induction with implications for the optimal timing of biologic therapy.

Keywords: Interleukin-6, CD4+ T-cell, Early rheumatoid arthritis

P43

Christine Basmadjian

Title: Design and synthesis of kinases irreversible inhibitors

Northern Institute for Cancer Research (NICR)

The objective of this project is to develop an irreversible inhibitor that targets a specific cysteine of a kinase of interest involved in cancer. This is aiming to improve selectivity towards the active site of the targeted protein.
An initial set of acrylamides added on a flexible linker resulted in a significant loss in potency and showed no evidence of covalent binding. We hypothesised that the loss in potency observed with acrylamide warheads was due to the amide functionality not being tolerated within the back pocket.
Accordingly, a series of alternative reactive groups were designed and synthesised. The incorporation of an acetylene substituent gave an increased potency of 0.19 μM.
Future medicinal chemistry work will further explore the structure-activity and reactivity properties of analogues to increase non-covalent affinity / selectivity.

Keywords: Cancer Drug Discovery, Medicinal Chemistry, Inhibitor

P44

Katherine Johnson

Title: Targeted exome sequencing to detect causative variants in 1,001 patients with unexplained limb-girdle weakness

Institute of Genetic Medicine (IGM)

Genetic muscle diseases are a heterogeneous group of rare disorders that are often characterised by progressive proximal skeletal muscle wasting and weakness. Worldwide collaborations and data sharing are essential to afford the power necessary to decipher rare muscle disease aetiology. We describe here the conclusion of the first phase of the MYO-SEQ project, an international research collaboration that applied targeted whole exome sequencing (WES) to the largest ever cohort of patients with undiagnosed proximal muscle weakness. We aimed to facilitate a clearer understanding of disease aetiology, enhance the diagnostic pathway and heighten the awareness of neuromuscular disorders – particularly limb-girdle muscular dystrophies (LGMDs). Forty-three neuromuscular disease referral centres from throughout Europe and the Middle East submitted 1,001 patients who presented with limb-girdle weakness and/or elevated creatine kinase activity. WES was performed using Illumina exome capture and a Picard-based processing pipeline. The variant call set was uploaded onto the Broad Institute of Harvard and MIT's seqr platform and 429 muscle disease-associated genes were examined. Likely pathogenic variants were identified in 52% of patients, with mutations identified in 85 known disease-associated genes. LGMD2A (CAPN3), LGMD2B (DYSF), LGMD2L (ANO5) and Duchenne/Becker muscular dystrophy (DMD) were the most common in our cohort, together accounting for more than a third of these patients. We also detected copy number variants (CNVs) in 27 (3%) patients. Importantly, over 150 (15%) patients harboured mutations in genes that are associated with treatable or manageable conditions: for example, COLQ (CMS5), GAA (Pompe) and GFPT1 (CMS12). Implementing WES has significantly enhanced the capacity of standard clinical work-ups. We highlight the benefit of international collaborations – directly to the patient and to the rare disease research community. Overall, our study has pioneered an accessible diagnostic pathway to expedite future diagnoses for neuromuscular disorders.

Keywords: muscle disease, exome sequencing,

P45

Stephanie Meyer

Title: Investigating the potential genotoxic effects of ionising radiation on BRCA1/BRCA2 mutant cells

Institute of Cellular Medicine (ICM)

Ionising radiation (IR) has multiple clinical applications, ranging from imaging to therapy. Whilst there are clear benefits associated with the use of medical radiation, it is important to be fully aware of any potential adverse effects. IR can cause mutagenic DNA double strand breaks (DSBs) which may lead to cancer. The genotoxic effects of IR depend upon exposure levels as well as inter-individual variation in DNA repair capacity. Some individuals have defects in the DNA damage response pathways (e.g. due to hereditary/sporadic mutations in DNA damage repair genes) and are likely to be more susceptible to genotoxicity after IR.
This study will investigate whether IR causes higher levels of DNA damage in cells with heterozygous mutations in the BRCA1 and BRCA2 genes, which are associated with defective DNA repair and may lead to breast and ovarian cancer. A panel of cells with defined BRCA1/BRCA2 mutations and control cells will be exposed to therapeutically relevant doses of IR. The formation/repair of DNA DSBs will be quantified (γ-H2AX and COMET assay) and effects on chromosome structure investigated (micronucleus assay). Initial data show that there is no difference in DNA damage levels/genomic instability in cells with a mutation that results in hemizygous BRCA1 knock-out compared to control cells following exposure to 2Gy. Studies will be expanded to include other clinically relevant BRCA1/BRCA2 mutations.
Results from this study will increase the understanding of potential genotoxic effects of IR in BRCA1/BRCA2 carriers and help to inform the safe/appropriate use of medical radiation in clinical scenarios.

Keywords: Ionising radiation, DNA damage, BRCA1/2

P46

Rachael Lawson

Title: Defining delirium in idiopathic Parkinson's disease: a systematic review

Institute of Neuroscience (IoN)

Background:
Parkinson's disease patients may be at increased risk of delirium and developing adverse outcomes, such as cognitive decline and increased mortality. Delirium is an acute confusional state that has overlapping symptoms with Parkinson's and dementia, making it difficult to identify. This study aimed to determine the diagnostic criteria, prevalence, management strategies and outcomes of delirium in Parkinson's through a systematic review of the literature.
Methods:
Seven databases were used identify all articles published before February 2017 comprising two key terms: "Parkinson's Disease" and "delirium". Data were extracted from studies meeting predefined inclusion criteria.
Results:
Twenty articles were identified. Delirium prevalence in Parkinson's ranged from 0.3-60% depending on setting; a diagnosis of Parkinson's was associated with an increased risk of developing delirium. Delirium was identified/diagnosed using seven different criteria. Symptoms included confusion, disorientation, hallucinations and/or delusions, but their frequency was not assessed or reported. Delirium may be associated with an increased length of hospital stay and worsening motor symptoms. We did not identify any studies examining the management of delirium in Parkinson's.
Discussion:
This review highlights the paucity of well-designed, appropriately powered studies investigating delirium in Parkinson's. The results suggest that delirium is a significant issue in people with Parkinson's and that having Parkinson's may be a risk factor for adverse outcomes, particularly in inpatient settings. Further prospective research is needed to accurately determine the prevalence of delirium in Parkinson's, its management strategies and outcomes, and to evaluate diagnostic criteria to differentiate between the overlapping symptoms of Parkinson's and delirium.

Keywords: Parkinson's disease, delirium, prevalence, systematic review

P47

Lina Hamadeh

Title: An integrated cytogenetic and genomic classifier in paediatric acute lymphoblastic leukaemia, a validation study

Northern Institute for Cancer Research (NICR)

Chromosomal and genetic alterations are hallmarks of Leukaemia. Recent genome-wide analysis studies improved our understanding about these alterations and their response to treatment. Our group previously developed a 3-tier risk classification based on the copy-number alteration (CNA) data of the 8 most commonly deleted loci in acute lymphoblastic leukaemia (ALL) using data on 809 patients treated on clinical trial, UKALL97/99. The Good-risk CNA features included normal copy-number status for all 8 genes, isolated deletions affecting ETV6/PAX5/BTG1 and ETV6 deletions with single additional deletion of BTG1/PAX5/CDKN2A/B. The Poor-risk CNA features included isolated deletions affecting IKZF1/PAR1/RB1 and the simultaneous deletion of IKZF1/PAX5/CDKN2A/B. The Intermediate-risk CNA features corresponded to the remaining CNA profiles. We then, integrated this risk classification with the established cytogenetic system to generate a refined 2-tier genetic risk classification. Although we validated the developed classifications on 742 UKALL2003 patients, there was still a need to validate them on a larger cohort of patients, followed over a longer period of time. For this, we used data on 3,239 B-cell precursors ALL patients collected from 12 different clinical study groups.
A total of 106 unique CNA profiles were observed in the validation cohort with 49 profiles not observed in the original cohort. Integrating the CNA with the cytogenetic risk showed that there is 4-tier genetic risk classification with different event free survival (EFS 91% vs 86% vs 73% vs 54%). This classification identified a small subgroup of patients (2%) who had good risk cytogenetic features but did not respond well to the given treatment. Agreeing with our previous study, this classification identified the patients with no established cytogenetic results who had improved EFS than the remaining patients in this subgroup (86% vs 73%). In conclusion, the genetic risk classification should be considered for treatment stratification in future clinical trial.

Keywords: Acute lymphoblastic leukaemia, Risk classification

P48

Bas Olthof

Title: Nitric oxide modulates NMDA-receptor neurotransmission in the auditory pathway.

Institute of Neuroscience (IoN)

Neuronal nitric oxide synthase (nNOS) catalyses the synthesis of the gaseous neurotransmitter nitric oxide (NO). Due to its gaseous nature, NO exhibits unique properties and is not constraint to the synaptic environment. NO modulates neural excitability by influencing ion channels via a pathway involving the downstream target soluble guanylate cyclase (sGC).

Here we demonstrate that nNOS is highly expressed and functionally active in the inferior colliculus (IC), the main midbrain centre in the auditory pathway for processing ascending sensory information and feedback from the cerebral cortex.

We employed fluorescent immunohistochemistry in brain sections from guinea pig to study underlying protein interactions between nNOS and proteins related to glutamate neurotransmission mediated by NMDA receptors (NMDA-R).

In addition to the previously reported cytoplasmic expression of nNOS, we observed many neurons in the IC expressing nNOS in puncta on their membranes. Further image analysis with Imaris, investigating these new found puncta, demonstrated that these nNOS puncta virtually always co-occurred with NMDA-R and sGC(α2) and the postsynaptic scaffolding protein, PSD95.

Electrophysiological recordings of neural activity were made in the IC of anaesthetised guinea pigs in response to sounds. Drugs targeting NO signalling were applied by microdialysis via probe inserted in the IC near the recording electrode.

The perfusion of NMDA into the IC caused a dose-dependent increase in the neuronal firing elicited by sounds. Remarkably, this increased response to NMDA was abolished in the presence of an nNOS inhibitor, L-methyl arginine, or ODQ, a drug that blocks the binding of NO to sGC.

Our results suggest that NO is an important modulator of glutamatergic NMDA-R activity in the IC, and that the functional dependence of NMDA-R activity on NO occurs via a multi-protein signalling complex consisting of NMDA-R, nNOS, and sGC held together by PSD95.

Keywords: nNOS Glutamate NMDA Auditory

P49

Rachel Crossland

Title: Mesenchymal Stromal Cell-Derived Extracellular Vesicles Show Distinct Chondrogenesis MicroRNA Expression Profiles from their Parental Cells

Institute of Cellular Medicine (ICM)

Mesenchymal stromal cells (MSCs) are frequently used in clinical trials for diverse immunological and degenerative diseases. The key clinical benefit of MSCs may be attributed to their paracrine factors, including MSC-secreted extracellular vesicles (MSC-EVs), indicating their potential as a cell-free therapy for regenerative medicine. However, the role of MSC-EVs in MSC biology is largely unknown and their molecular composition has not been fully characterised. Here we report the microRNA expression profiles of MSC-EV, including chondrogenesis microRNAs.

Primary BM-MSC (n=3) identity was determined by phenotypic profiles, morphology and tri-lineage differentiation. MSC-EVs (n=3) were isolated from cell-conditioned medium by differential ultracentrifugation, and characterised by flow cytometry (CD83/CD63/CD9), western blot (Alix&Flotillin), NTA and electron microscopy. Global microRNA expression profiling was performed using NanoString Human MicroRNA V3 (n=799) and selected microRNAs were assessed by qRT-PCR.

Comparing matched MSC and MSC-EV samples, 50 microRNAs were significantly differentially expressed (fold change (FC) -49.04-85.93, p-value <0.001-0.049). Of these, 39 were downregulated (FC -1.96--49.04, p=<0.001-0.049) and 11 were upregulated (FC 1.71-85.97, p=0.001-0.047) in MSC-EVs. The top 5 highly expressed microRNAs comprised >50% of total expression counts (MSCs=51.8%, MSC-EVs=71.3%). qRT-PCR validation in an independent cohort (n=7) confirmed 4 chondrogenesis microRNAs to be over expressed in MSC-EV vs. MSC (miR-29b p=0.01, miR-142-3p p<0.001, miR-21-5p p=0.004, miR-140 p=0.02), and miR-145-5p which was under-expressed in MSC-EV vs. MSC (p=0.04). Co-culture of a chondrocyte cell line (T/C28a2) with MSC-EVs resulted in improved wound closure in a standard scratch assay.

MSC-EVs show differential expression of specific microRNAs, including chondrogenesis-related microRNAs from parental MSCs, which may contribute to their clinical benefit. This has implications for cell-free therapies for degenerative cartilage diseases, including osteoarthritis.

Keywords: Exosome, MicroRNA, MSC, osteoarthritis, chondrogenesis

P50

Abeer Dannoura

Title: GREB1 and PDZK1 predict oestrogen responsiveness in gynaecological cancer

Northern Institute for Cancer Research (NICR)

Background: Gynaecological cancer contributes to considerable female cancer-related mortality. Endocrine therapy for metastatic gynaecological cancer has potential in endocrine-sensitive tumours. Despite, a promising response in a subgroup of oestrogen receptor alpha -positive patients, a variable benefit has been demonstrated. In addition, an oestrogen response has been obtained in some cases of oestrogen receptor alpha-negative gynaecological cancers. Additional oestrogen-sensitive predictive biomarkers might allow clinicians to select more accurately women who will benefit. Our aim is to identify and validate oestrogen-responsive predictive biomarkers.
Methods: The proliferative response to 17β-oestradiol of twelve gynaecological cancer cell lines, from five ovarian and three endometrial cancers, two granulosa cell tumours, and two endometrial stromal sarcomas was assessed. The effects of 17β-oestradiol on the expression of known oestrogen-responsive genes was analysed in parallel by western transfer.
Results and conclusion: A robust proliferative response to oestrogen was obtained in two ovarian and one endometrial cell line. Other cell lines that express oestrogen receptor alpha did not respond. Most of the known oestrogen response genes were either not regulated by oestrogen or were regulated in all gynaecological cancer cell lines.
Of the known oestrogen responsive genes, regulation of GREB1 and PDZK1 was associated most closely with proliferative response to oestrogen.

Keywords: GREB1, PDZK1, Gynaecological cancer, oestrogen

P51

Marco Salamina

Title: Role of Cyclin A and Cyclin E in p27 turnover through the SCF-Skp2 complex

Northern Institute for Cancer Research (NICR)

Cell cycle is driven by a consequential activation of Cyclin Dependent Kinases (CDK). The passage between G1 and S phase is orchestrated by the CDK2 that binds Cyclin E and then Cyclin A that hold the cell in the DNA synthesis phase. The mechanism that provide the switch between these two cyclins is poorly understood, although it is frequently altered in a variety of cancer cells. The tumor suppressor gene p27KIP inhibits both CyclinA-CDK2 and CyclinE-CDK2 complexes. The SCF-Skp2 complex provide the turnover of p27KIP. We have identified the molecular structure of a novel binding site for Skp2 on Cyclin A, that it is not present in Cyclin E and that is mutually exclusive with p27KIP. Moreover, we analyzed the role of Cks1 during the assembly with the SCF-Skp2 complex and its role during the ubiquitination of p27KIP. Hence, these results explain the role of the interaction between Cyclin A and Skp2 during the cell cycle progression through the degradation of p27KIP.

Keywords: CDK SCF-SKP2 CELL CYCLE

P52

Urszula Cytlak

Title: Ikaros family zinc finger 1 regulates dendritic cell development and function in humans

Institute of Cellular Medicine (ICM)

The prototypic functions of dendritic cells (DCs) include the polarization of naïve T-cells or induction of tolerance, but they also regulate a range of leukocyte responses. The diverse functions of DCs are represented by at least three subsets, conventional DC1 (cDC1), cDC2 and plasmacytoid DC (pDC). The latter have been shown to support B-cell proliferation, memory differentiation and immunoglobulin secretion and are implicated in the pathogenesis of multiple myeloma.
Haematopoietic cell development and lineage specification is coordinated by transcription factors (TF), which may govern the expression of both differentiation and functional genes. Ikaros family zinc finger 1 (IKZF1) is a TF required for mammalian B-cell development. IKZF1 deficiency also reduces pDC numbers in mice but its effects on human DC development are unknown.
Heterozygous IKZF1 mutations in humans were recently identified as the cause of an immunodeficiency syndrome characterized by progressive loss of B-cells, hypogammaglobulinaemia, T-cell subset skewing, recurrent infections and autoimmunity. IKZF1 protein is also targeted for selective proteosomal degradation by the drug lenalidomide, used to treat myeloma.

Enumeration, phenotypic and functional analyses were performed on peripheral blood monocyte and DCs in twenty affected individuals from four kindreds with heterozygous IKZF1 mutations. Similar analyses were undertaken in cells with reduced IKZF1 protein levels resulting from in-vivo or in-vitro exposure to lenalidomide.

Loss of pDC, expansion of cDC1s and reduction in non-classical monocytes were consistent findings in patients with IKZF1 deficiency. pDC loss was replicated in myeloma patients and in-vitro cultures treated with lenalidomide. DC functional assays revealed a reduction in subset specific cytokine production in the context of genetic or pharmacological IKZF1 deficiency.

IKZF1 has an essential role in human DC development and function. The DC defects in IKZF1 deficiency may contribute to the immunodeficiency in these patients but augment the therapeutic benefit of lenalidomide treatment in myeloma.

Keywords: dendritic cells, IKZF1, immunodeficiency, human haematopoiesis

P53

Dennis Lendrem

Title: Do Scientists Do Science?

Institute of Cellular Medicine (ICM)

This study reports on preliminary data from the Oxford Project: a 50 year longitudinal study drawing upon field observations and experimental studies of scientific behaviour in Homo sapiens. We describe 'world class' scientific behaviours and evaluate the extent to which these behaviours are observed in both academic and industry research. In particular we focus on research behaviours in the medical sciences.

Keywords: science medicine research

P54 (poster withdrawn)

Sian Russell

Title: Qualitative systematic review of self-management of COPD: the views of patients and healthcare professionals

Institute of Health and Society (IHS)

Self-management interventions for chronic obstructive pulmonary disease (COPD) can improve quality of life, reduce hospital admissions, and improve symptoms. However, many factors impede engagement for patients and practitioners. Qualitative research, with its focus on subjective experience, can provide invaluable insights into such factors. Therefore, a systematic review and synthesis of qualitative evidence on COPD self-management from the perspective of patients, carers, and practitioners was conducted. Following a systematic search and screening, 31 studies were appraised and data extracted for analysis. This review found that patients can adapt to COPD; however, learning to self-manage is often a protracted process. Emotional needs are considerable; frustration, depression, and anxiety are common. In addition, patients can face an assortment of losses and limitations on their lifestyle and social interaction. Over time, COPD can consume their existence, reducing motivation. Support from family can prove vital, yet tinged with ambivalence and burden. Practitioners may not have sufficient time, resources, or appropriate skills or confidence to provide effective self-management support, particularly in regard to patients' psychosocial needs. This can compound patients' capability to engage in self-management. For COPD self-management to be effective, patients' psychosocial needs must be prioritised alongside medication and exacerbation management. In addition, patients' personal beliefs regarding COPD and its management should be reviewed periodically to avoid problematic behaviours and enhance positive adaptions to the disease. Patients with COPD are not a homogenous group and no one intervention will prove effective for all. Finally, practitioners require greater education, training, and support to successfully assist patients.

Keywords: Qualitative; self-management; COPD; chronic conditions

P55

Simon Ramsbottom

Title: Murine models and patient exome sequencing identify a novel modifier locus for cystic kidney disease

Institute of Genetic Medicine (IGM)

Nephronophthisis (NPHP) is responsible for up to 25% of childhood end stage renal disease (ESRD). It characterised by tubular basement membrane disintegration and atrophy, formation of cortico-medullary cysts and interstitial fibrosis. NPHP is a ciliopathy and may often form one part of a multi-system disorder. While the progression of disease often takes place over a number of years, there is a large degree of heterogeneity of kidney disease phenotype between patients, even with the same genotype.
To model NPHP and the variation observed in patients we used a Cep290 gene trap murine model of NPHP, which varies dramatically depending on the strain of mouse in which the mutation is propagated. By interbreeding two strains we generated homozygous Cep290 gene trap F2 mutants and performed a genome wide SNP array in order to identify a modifier locus. An 11MB region of the mouse genome was identified which contained alleles associated with rapid development of kidney cysts. Whole exome sequencing of patients sharing a common mutation in CEP290, but with varying phenotypes, was used to confirm putative modifier alleles giving rise to more severe kidney phenotypes. These data indicate that the extent of kidney involvement in ciliopathies is genetically determined and that identification of risk alleles associated with rapid disease progression may in future be used to inform patient management.

Keywords: kidney, nephronophthisis, ciliopathy, rare disease

P56

Rong Ou

Title: Treg activation by CD40 activated B cells

Institute of Cellular Medicine (ICM)

Background: CD4+FoxP3+ regulatory T cells (Treg) play important in control T lymphocytes activation and prevent autoimmunity. Activated Treg express Tbet, and Tbet expressing Tregs are essential to suppress Th1-driven autoimmune diseases. Little is known about how Tregs are activated and what type of antigen presenting cells activates Tregs. While DC plays important role in activating T conventional cells (Tconv), B cells also can be good antigen presenting cells when they are activated to express high level of MHC class II and CD80/86 costimulatory molecules. This study aims to understand the role of CD40 activated B cells in Treg activation.
Methods: Splenocytes were isolated from naïve mice and CD8 T cells were depleted using magnetic sorting by negative selection. Cells were plated in 48 well plates with 4 million cells per well. Anti-CD40 and anti-B cell receptor (BCR) antibodies were used to stimulate B cells. B cells and T cell activation was measured by flow cytometry after 3 days stimulation. Cell proliferation was measure by ethynyl deoxylunridine incorporation using flow cytometry.
Results: Anti-CD40 and BCR synergistically activated B cells. Activated B cells expressed high level of MHC class II and CD80/86. B cell stimulated with anti-CD40 and BCR efficiently activated syngeneic Treg, induced Treg proliferation, and induced Tbet and CD69 expression in Tregs, but not in conventional CD4 T cells (Tconv). Anti-CD40 stimulation also induced IFNγ production in the culture, and Tbet expression in Treg is not dependent on IFNγ.
Conclusion and discussion: Our findings support that activated B cells selectively activate syngeneic Treg, but not Tconv. This study suggests that B cells may play an important role to main immune homeostasis when activated to prevent autoimmune disease progression.

Keywords: Treg, CD40, BCR, B cells, Tbet

P57

Flint Stevenson-Jones

Title: Direct interactions between the bacterial RNAP and ribosome

Institute of Cell and Molecular Biosciences (ICaMB)

Transcription and translation form the basis of gene expression in all living organisms. In prokaryotes they occur within the same cellular space and at the same time. The ribosomes begin translation of the RNA as soon as the newly synthesised RNA emerges from RNA polymerase (RNAP), a process known as coupling. Interplay between the two machineries is highly complex and plays an important role in gene expression. Coupling between transcription and translation has been shown to influence the transcription elongation rate, prevent premature transcription termination and has also been implicated in the maintenance of genome integrity. The molecular principles of transcription-translation coupling are however poorly understood. Throughout the cell cycle both transcription and translation are subject to regulation from accessory factors making in vivo study of any direct interactions difficult. An in vitro transcription-translation system developed in our lab made up of pure components required for transcription and translation allows stepwise control of the RNAP and the ribosome and allows us to examine direct interactions without interference from any accessory factors normally present within the cell, as well as investigate the roles of individual factors in coupling. Using this technique we were able to determine how close the coupled ribosome can approach the RNAP with single nucleotide precision. We discovered that the ribosome was able to approach RNAP closer than previously thought based on footprinting and structural data. During transcription RNAP is prone to moving backwards along the DNA template but we show that the approaching ribosome is able to inhibit this backwards movement of the RNAP. Altogether our results reveal a very close interaction between the RNAP and the ribosome that has potential implications during regulation of gene expression.

Keywords: Transcription, translation, bacteria

P58

Akin Cayir

Title: RNA N6-methyladenosine (m6A) Methylation Machinery Genes Expression in Human Cancers

Institute of Cellular Medicine (ICM)

Today, more than 100 RNA modifications have been known in various organisms and most of them have been found in different RNA types including ribosomal RNA, transfer RNA, small nucleolar RNA, and messenger RNAs. Recent findings from transcriptome-wide studies cleared that adenine-6 methylation (m6A) is the most common and prominent modifications in mRNAs in eukaryotic organisms. m6A is the first modification which has been characterized with writers, erasers, and readers proteins in humans. Althoug m6A has shown curicial roles on wide range of biological functions, its role in cancer biology remains largely unknown. Therefore, in the present study, we analyzed the RNA sequencing expression data (writer, erasers, and readers proteins of m6A) of 9,736 tumors and 8,587 normal samples from the The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects. We observed that expression of writer gene was significantly lower in 8 different tumor types. On the other hand, the expression of writer gene was significantly higher in two tumor types. The expression of two eraser genes were significantly higher in 6 different types of tumor, and significantly lower in three types of tumors. Likewise, the expression of readers were significanly higher in eight type of tumors. To sum up, by analyzind the data, we observed that m6A RNA methylation involved in different types of tumor. Thus, m6A modification has potential to be a candidate of biomarker and a new therapeutic target in various tumors.

Keywords: RNA methylation, N6-methyladenosine (m6A) , cancer

P59

Peixun Zhou

Title: Investigation of FOXO1 Abnormalities in Paediatric Burkitt Lymphoma

Northern Institute for Cancer Research (NICR)

Burkitt lymphoma (BL) is an aggressive mature B cell non-Hodgkin lymphoma. Although high cure rates are achieved for children with BL by intensive therapy in the western world (sporadic BL, sBL), survival is still poor in resource-constrained countries such as Malawi (endemic BL, eBL), and risk of relapse remains high. Also treatment is highly toxic with severe side effects. Thus markers are needed to identify patients with high risk of relapse and to reduce treatment-related toxicity. FOXO1 is highly expressed in normal germinal center B cells, BL cell lines and primary samples. However, role of FOXO1 in BL is not clear.

Paediatric sBL and eBL cohorts are being established in our lab. Copy number were detected by Affymetrix arrays. FOXO1 mutations were revealed by Sanger sequencing, and FOXO1 protein was knocked down/out by CRISPR/Cas9.

Gain of 13q14.11 (including FOXO1) was detected in 20.6% of eBL and 8.0% of sBL cases, with a hazard ratio for risk of relapse at 2.69 (p=0.059) in eBL. Although not statistically significant, it demonstrates FOXO1 gain is strongly associated with higher risk of relapse in eBL. In addition, FOXO1 non-synonymous mutations in 46.6% of our eBL cohort and in 23.9% of sBL cohort, significantly higher than in literature. Majority of recurrent FOXO1 mutations in this study disrupt the AKT recognition motif of FOXO1, potentially leading to nuclear accumulation of FOXO1 (activation). Moreover, absence of FOXO1 by CRISPR/Cas9 significantly reduced proliferation of BL cell-line cells.

Association of FOXO1 gain with increased risk of relapse, presence of high frequency of potential FOXO1 activating mutations, and reduction of cell proliferation in the absence of FOXO1 demonstrate an oncogenic role of FOXO1 in paediatric BL. We are currently screening more samples and will further investigate if FOXO1 can be targeted therapeutically.

Keywords: Burkitt lymphoma, FOXO1

P60

Henrique de Paula Lemos

Title: Dissecting the immunoresponses induced by systemic activation of the Stimulator of Interferon Genes (STING).

Institute of Cellular Medicine (ICM)

The DNA sensor cGAMP Synthase converts cytosolic DNA into cyclic-GMP-AMP that binds to the Stimulator of Interferon Genes (STING). STING activation stimulates TBK1 to phosphorylate IRF3, thereby inducing transcription of type I IFNs. Simultaneously, STAT6 and NF-κB are also activated, inducing the production of pro-inflammatory cytokines, e.g., TNF-α, IL-6, and IP-10. Most studies evaluating the pathways activated by STING have been performed in cell lines in vitro, and little is known about how these pathways interact with each other in vivo to promote different types of immunoresponses. In this study, we evaluated cytokine production in spleens after single and multiple intravenous injections of STING agonists, DNA nanoparticles (DNPs) and cycled di nucleotides (CDNs), in mice. The results show that the production of TNF-α, IL-6, IFN-γ, IL-10 and CXCL10 in spleens induced by a single injection of DNPs and CDNs was reduced or completed ablated in absence of type I IFN signalling. After 5 injections with DNPs, TNF-α and IL-10 were not detected, IL-6 reduced, and the levels of IFN-γ and CXCL10 not changed in spleen when compared with the levels observed with a single injection of DNPs. In absence of type I IFN signalling, multiple injections with DNPs restored and boosted the production of TNF-α and IL-6/IFN-γ, respectively. These results reveal a crucial dependency of type I IFN for the induction of NFkB-driven cytokines after a single injection of STING activators, suggesting that after STING activation in vivo NFkB and IRF3 pathways are interdependent. Moreover, the boosted NFkB-driven cytokine production after multiple injections suggest that type I IFN plays a regulatory role in the inflammation induced by STING activation, which could be have an impact on the efficacy of STING agonists when clinically used.

Keywords: STING, Interferons, NFkB, cytokines, IRF3, immunoregulation

P61

Tamilvendhan Dhanaseeian

Title: Evaluation of the zebrafish desmin gene duplication event

Institute of Genetic Medicine (IGM)

Background: Mutations in the type III intermediate filament, desmin, are associated with limb-girdle muscular dystrophy and cardiomyopathy. The mechanism of the late onset myopathy and variable cardiac phenotype remain unclear. Although there are a limited number of transgenic and knockout mice models, there is a need for a tractable in-vivo laboratory model to investigate these aspects. The ease of genetic manipulation and the vertebrate body plan of the zebrafish suggests that this would be a candidate system. However, zebrafish have undergone a genome duplication event, which complicates their use.
Aim: We set out to evaluate the effect of genome duplication on the expression of the desmin gene in zebrafish.
Method: The desmA and desmB transcriopts were cloned and compared to human desmin. We have mapped the expression pattern of the desmin A (desmA) and desmin B (desma) genes through development and in adult fish using RT-PCR and in-situ hybridisation. Desmin A and Desmin B mutant lines have been generated by CrispR-Cas9 genome editing.
Results and Conclusion: We demonstrate differential expression and co-expression of the two desmin gene products during development and in adult zebrafish. Importantly, the differential expression of these genes indicates that it should be possible to create separate models of human disease in either skeletal or cardiac muscle cells by targeting one of the two genes.

Keywords: Zebrafish, cardiac, skeletal muscle

P62

Julie Worrell

Title: c-Rel is a Master Regulator of Fibroblast Phenotype in Pulmonary and Dermal Fibrosis

Institute of Cellular Medicine (ICM)

Objectives: c-Rel is a member of the nuclear-factor-kappa-B family of transcription factors. NF-κB signalling regulates genes controlling inflammation, cell proliferation, survival and matrix production. Dysregulation of this pathway alters normal skin physiology. This study investigates if c Rel stimulates the fibrosis promoting activities of fibroblasts in the context of systemic sclerosis.

Methods: RNA sequencing and pathway analysis was performed on murine lung and dermal fibroblasts. Microarray analysis was performed on publically available datasets. Serum levels of matrix proteins were examined in SSc patients. c-Rel binding to promoters in fibroblasts was assessed by chromatin immunoprecipitation. Inhibition (siRNA) and over-expression (transient transfection) of c-Rel was investigated in human dermal fibroblasts. Functional assays examined fibroblast proliferation, migration and matrix production. Mice were challenged with bleomycin to induce chronic lung or skin fibrosis.

Results: c-Rel expression is elevated in chronic murine fibrosis models and SSc. c-Rel-/- mice were protected from pulmonary fibrosis. Fibroblasts from c-Rel /- mice displayed significant increases in proliferation and migration with decreased soluble collagen production. c-Rel regulates matrix genes involved in extracellular structure, matrix and supramolecular fibre organisation. An NF-κB gene signature was identified in diffuse scleroderma skin. c-Rel directly binds the Col1a1 and Fn promoters. c-Rel knockdown in primary human dermal fibroblasts significantly decreased expression of matrix turnover genes. Fibronectin was significantly elevated plasma of SSc patients.

Conclusions: c-Rel regulates a fibrogenic transcriptional programme in fibroblasts with a fundamental mechanistic role in disease pathology. Targeting c-Rel in fibroblasts of patients with SSc may supress fibrogenic activity.

Keywords: c-Rel; fibrosis; fibroblasts; extra-cellular matrix scleroderma

P63

Martina Finetti

Title: A strategy for Pre-Clinical Therapeutic Target Identification in the Absence of Actionable Mutations

Northern Institute for Cancer Research (NICR)

ATRTs have low mutation rates and few classically-actionable variants to direct molecularly targeted therapies; loss of SMARCB1 is the sole recurrent mutational event in >90% of ATRTs. We have designed and implemented a genome-scale strategy for target identification and prioritization in tumors devoid of significant actionable mutations. Re-expression of SMARCB1 causes ATRT cells to cease proliferation and differentiate; we therefore hypothesized that identifying and counteracting critical downstream SMARCB1-dependent events represents a primary route to therapeutic intervention.
We identify such events using an integrated genome-wide approach encompassing genome-scale CRISPR/Cas9 functional screens (GeCKO screening; 122,411 sgRNAs) alongside expression/DNA methylation profiling of primary ATRTs and ATRT cells following SMARCB1 re-expression and/or treatment with demethylating agents. Cross-referencing these analyses, we use a rational selection algorithm, to identify critical tumorigenic genes/pathways and proceed to validate their ability to be targeted as therapeutic targets.
Our strategy identifies, ranks and prioritizes multiple SMARCB1-dependent pathways/genes functionally essential to ATRT, and characteristic of the primary tumour; including those previously described (Rb/CDK4/6, SHH, MYC), those less well evidenced (mTOR, TGF-β, HGF, Stat3/Jak) and novel SMARCB1-dependent synthetic lethalities (NuA4 complex, PIM1). We also describe the repressive genome-wide effect of SMARCB1 mutation on the transcriptome, through altered SWI/SNF binding, associated histone marks and localized hypermethylation and the therapeutic possibilities implied therein.
Data and web-based interactive analysis and visualization tools will be made publically available to help guide and benchmark future pre-clinical testing in ATRT. This study pinpoints SMARCB1-dependent therapeutic susceptibilities in MRTs with the capacity to inform future treatment strategies.

Keywords: Pediatric cancer; pre-clinical target identification

P64

Fiona Malcomson

Title: Adherence to the WCRF/AICR cancer prevention recommendations and WNT

pathway-related markers of bowel cancer risk .

Institute of Cellular Medicine

Abnormal WNT signalling is implicated in the aetiology of colorectal cancer (CRC), including abnormal WNT gene expression, and can lead to dysregulated cell proliferation. Most CRCs are sporadic and risk is strongly influenced by lifestyle factors e.g. diet. The WCRF/AICR cancer prevention recommendations include maintaining a healthy body weight and limiting intakes of red meat and processed foods. This study aimed to investigate the relationships between adherence to these recommendations and WNT pathway-related markers of CRC risk.

This study used dietary and lifestyle data from 75 healthy participants recruited to the DISC Study. A total adherence score (0-8) was derived from scores for seven of the recommendations plus smoking status. Expression of WNT pathway genes and regulatory microRNAs by qPCR, SFRP1 methylation by pyrosequencing, and crypt cell proliferation were assessed in colorectal mucosal biopsies. Relationships between total adherence score and the measured outcomes were analysed using Spearman's rank correlation analysis. Unpaired t-tests were used to examine the effects of scores for individual recommendations.

The mean total adherence score was 3.2 (range 1-6). Total adherence score was inversely correlated with expression of c-MYC ($p=0.039$) and WNT11 ($p=0.025$). Adherence to the recommendation on body fatness was associated with reduced expression of AXIN2 ($p=0.025$) and GSK3β ($p=0.027$). Decreased expression of CTNNB1 ($p=0.046$) and WNT11 ($p=0.034$) was observed in participants who met the recommendation for dietary fibre intake. Expression of the oncogenic miR-17 was 20% lower in these participants ($p=0.030$) and also reduced in those adhering to the recommendation for fruit and vegetable intake ($p=0.035$). The findings from this study provide evidence for positive effects of adherence to the WCRF/AICR cancer prevention recommendations on WNT pathway-related markers of CRC risk and suggest that these dietary factors act early in the tumorigenesis pathway.

Keywords: nutrition, cancer prevention, bowel cancer, lifestyle

P65

Nicola Maney

Title: Exploring PIM1 as a measurable therapeutic target in early rheumatoid arthritis

Institute of Cellular Medicine

Background: We previously showed the RNA levels of protein kinase PIM1 to be strikingly up-regulated amongst circulating CD4+ T cells of untreated early RA (eRA) patients compared with disease controls1. PIM1 has a recognised role in T cell development and is implicated in autoimmunity. Oral PIM kinase inhibitors are in clinical development in oncology; for example PIM1 was recently identified as a promising target in triple-negative breast tumours2. We hypothesised that, amongst an identifiable subgroup of eRA patients, PIM1 overexpression in CD4+ T cells is a targetable early event in pathogenesis.

Methods: Peripheral blood was obtained from consenting healthy donors or treatment- naïve eRA patients and CD4+ T cells were isolated by positive selection. PIM1 transcript knock-down (SMARTpool siRNA, Dharmacon) or protein inhibition (small molecule inhibitor TCS PIM-1, Tocris) was undertaken and flow cytometric analysis was used to assess its potential role in activation and proliferation following CD3/CD28-mediated stimulation.

Results: Both PIM1 knock-down and PIM1-specific inhibition decreased the activation and proliferation of stimulated healthy donor CD4+ T cells in vitro. Mirroring this finding in eRA, CD4+ T cells were also found to exhibit an activated, hyper-proliferative phenotype compared with those isolated from healthy donors, with significantly higher CD25 and ki67 expression. The production of pro-inflammatory cytokines was decreased by PIM1-specific inhibition but not knock-down potentially due to the involvement of other PIM family members.

Conclusions: Taken together, these data implicate PIM1 as prominent amongst genes whose induction “pre-programmes” CD4+ T cells to function aberrantly in disease. Conceivably, targeting PIM1 may regulate aberrant CD4+ T cell effector function in an identifiable subgroup of eRA patients, with fewer “off-target” effects than currently available IL-6 pathway-directed approaches. Ongoing work will explore this further.

Keywords: PIM1, Rheumatoid arthritis, T cells

P66

Rachel Dickinson

Title: Germline and somatically acquired ASXL1 mutations induce disease progression in GATA2

Institute of Cellular Medicine

Background

Previously we discovered that heterozygous GATA2 mutation is associated with a human immunodeficiency syndrome that we refer to as Dendritic Cell, Monocyte, B and NK Lymphoid (DCML) deficiency. The condition causes clinical problems including: sporadic monocytopenia and mycobacterial infection (MonoMAC); Emberger's syndrome and familial Myelodysplastic Syndrome (MDS)/Acute Myeloid Leukaemia (AML). We have identified 52 individuals with 27 different heterozygous GATA2 mutations within 33 pedigrees of DCML deficiency. Different families with the same GATA2 mutation can have varying clinical features. Within a family with GATA2 mutation associated traits can show variable penetrance. Research in other cohorts has suggested that the progression of GATA2 deficiency to leukaemia is associated with the acquisition of ASXL1 mutation.

Methods

Polymerase Chain Reaction (PCR) and Sanger Sequencing were used to screen our cohort of GATA2 mutated individuals and relatives for ASXL1 mutations. NanoString technology was used for gene expression analysis of lymphoblastoid cell lines and neutrophils from GATA2 deficient patients with and without ASXL1 mutation. Statistical analyses were performed using GraphPad Prism 7.

Results

ASXL1 mutations were detected in 15/37 (40%) patients with GATA2 deficiency. Sequencing of the DNA from non-haematopoietic cells or relatives implied that three of the ASXL1 variants were germline alterations. Two of these alterations were estimated to be deleterious using mutation prediction programmes. Principle component analysis of NanoString data indicated that GATA2 deficient individuals with ASXL1 mutation have a distinct gene expression profile from patients without ASXL1 mutation. Further analysis revealed that GATA2 deficient patients with ASXL1 mutation were more likely to: develop symptoms; be predisposed to HPV infections and progress to MDS at an earlier age ($P<0.05$).

Conclusion

The presence of a germline or somatically acquired ASXL1 mutation could enhance disease progression in patients with GATA2 deficiency. Therefore ASXL1 mutation could be a prognostic marker for GATA2 deficiency.

Keywords: GATA2, ASXL1, leukaemia, Immunodeficiency, genetics

P67

Kyle Thompson

Title: OXA1L MUTATIONS CAUSE MITOCHONDRIAL ENCEPHALOPATHY AND A COMBINED

OXIDATIVE PHOSPHORYLATION DEFECT

Institute of Neuroscience

OXA1, the mitochondrial member of the YidC/Alb3/Oxa1 membrane-protein insertase family, is required for the assembly of oxidative phosphorylation complexes IV and V in yeast. However, depletion of human OXA1 (OXA1L) was previously reported to impair assembly of complexes I and V only. We report a patient presenting with severe encephalopathy, hypotonia and developmental delay who died at 5 years showing complex IV deficiency in skeletal muscle. Whole exome sequencing identified biallelic OXA1L variants (c.500_507dup, p.(Ser170Glnfs*18) and c.620G>T, p.(Cys207Phe)), that segregated with disease. Patient muscle and fibroblasts showed decreased OXA1L and subunits of complexes IV and V. Crucially, expression of wild-type human OXA1L in patient fibroblasts rescued the complex IV and V defects. Targeted depletion of OXA1L in human cells or Drosophila melanogaster caused defects in the assembly of complexes I, IV and V, consistent with patient data. Immunoprecipitation of OXA1L revealed the enrichment of mtDNA-encoded subunits of complexes I, IV and V. Our data verify the pathogenicity of these OXA1L variants and demonstrate that OXA1L is required for the assembly of multiple respiratory chain complexes.

Keywords: OXA1L; insertase; mitochondria; OXPHOS; Complex IV; encephalopathy

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